SOME ASPECTS OF THE BIOLOGY AND DISTRIBUTION OF *Amphibola crenata* (GASTROPODA: PULMONATA) WITH SPECIAL REFERENCE TO POSSIBLE EFFECTS OF POLLUTION FROM SEWAGE OUTFALLS

A thesis

submitted for the Degree

of

Doctor of Philosophy in Zoology

in the

University of Canterbury

by

S.L. Bennington

University of Canterbury

1979

CONTENTS

ABSTRACT

Shell morphology and sculpture, population size structure, sediment grain size preference, and the reproductive cycle, are described for the common estuarine mud flat gastropod, *Amphibola crenata* (Martyn 1784). Snails which occurred near to sewage outfalls in the Avon-Heathcote Estuary, are compared with those which occurred in an area with similar salinity and exposure conditions but isolated from possible effects of sewage. Sediment silt and organic carbon content were higher near to the outfalls.

Shell morphology, described by shell dimensions, geometrical parameters of coiling, linear weight relationships and shell deposition patterns, showed no significant difference between the two areas. A predominantly juvenile population which occurred near to the sewage outfalls was the result of an annual cycle of settlement and subsequent population loss. A predominantly adult population occurred in the area unaffected by the outfalls. In laboratory experiments, animals from each area preferred sediment from their own particular area. Size frequency and sculpture distribution showed no regular zonation patterns for other areas of the estuary. Seasonal changes in the ovotestis were not significantly different between animals collected from the two areas.

Tissue levels of heavy metals in *A. crenata* collected from near to the sewage outfalls were lower per unit tissue dry weight but higher in total metal per individual than in those from the other area. Tissue and sediment levels of metals were relatively low in both areas compared with levels reported in studies of polluted conditions. Isolated areas of elevated metal levels occurred in the Avon River.

Exceptionally long survival times for *A. crenata* exposed to copper(II) ion concentrations in the laboratory, are attributed to pulmonary respiration, the operculum, and high mucous production. Salinities below 8% seawater and increase in temperature affected the toxicity of copper to adults. Veliger larvae were sensitive to much lower concentrations of ionic copper(II) (0.5ppm) than those which affected egg development (5ppm) or survival of adults (2.5ppm).

The size distribution of *A. crenata* near to the sewage outfalls does not seem to be a result of the presence of toxic materials in the sewage.

CHAPTER I

INTRODUCTION

1. THE PROBLEM

Until the early 1970s the sediment on the western intertidal slopes of the Avon-Heathcote Estuary, adjacent to the Christchurch Drainage Board sewage outfalls, was very muddy with a high water content (Knox and Kilner 1973). By 1974 the sediment in this area was firm and quite sandy (Macpherson 1977). Throughout the preceding period of almost twenty years, the pulmonate mud flat gastropod, *Amphibola crenata* was sparse in the immediate vicinity of these outfalls and increased in numbers along a transect away from this pollution source (Bruce 1953, Williams 1960, Rosenberg 1963, Kilner 1969). Unfortunately, this early work did not include detailed measurements of animal size, but Rosenberg (1963) observed the presence at the outfalls of a majority of "newly settled" individuals, less than 7mm diameter, whereas at Brighton Jetty (Fig.1.1) a full range of sizes, 7 to 29mm diameter, was found.

In 1974 my preliminary observations indicated that the apparently stable population of *Amphibola* on the western intertidal slopes adjacent to the sewage outfalls comprised a small proportion of large adults of shell length 23 to 26mm,

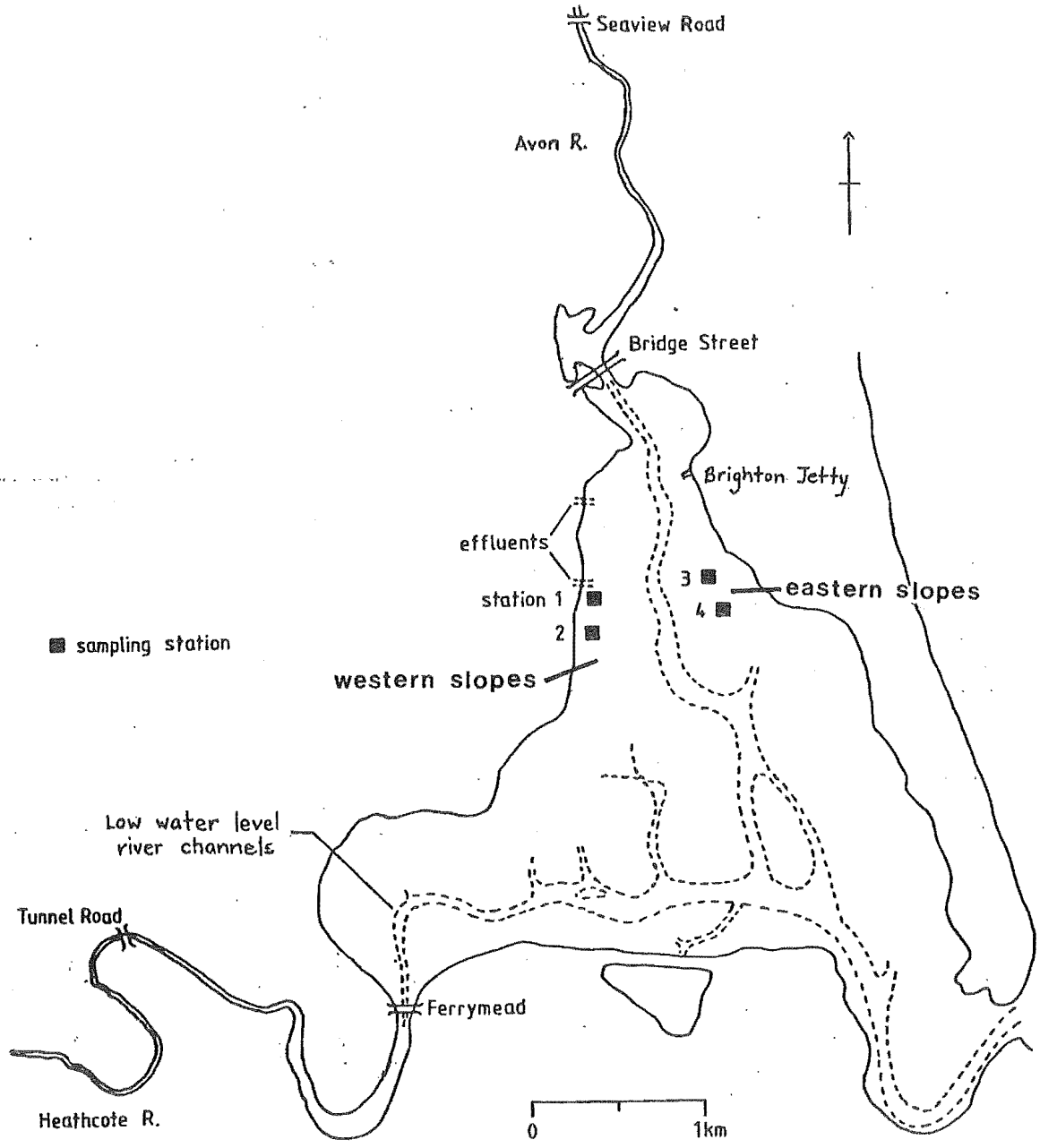


Fig.1.1. The Avon Heathcote Estuary (43°33'S, 172°44'E) showing the two study areas: the western slopes subject to sewage effluent, represented by stations 1 and 2; and the eastern slopes, on the opposite side of the Avon River channel, represented by stations 3 and 4.

and a large proportion of newly settled juveniles of shell length less than 4mm. In contrast, the population on the eastern slopes of the estuary in front of Jellicoe Park, opposite the sewage outfalls, conprised a dense distribution of slightly smaller adults of shell length 20 to 24mm, and a very small proportion of newly settled juveniles. I postulated that this difference in population structure may be a result of the combined effect of outfall-dominated sediment and chemical conditions in the immediate area of the outfalls. If this was the case then the existing sparse adult population adjacent to the outfalls may provide biological indications of stress produced by these environmental conditions. If recent (1974 to 1977) changes in sediment structure indicate changes in sewage outfall affect, then these new environmental conditions should be expressed subsequently in a changing *Amphibola* population structure in this area. It therefore seemed timely to compare the existing population in this area with that on the eastern slopes, and to monitor changes over a subsequent period, with respect to particular aspects of the biology of these two sub-populations. In this way an indicator of stress from the affects of sewage, related to the difference in population structure, may be identified for application to predicting biological responses to other similar environmental conditions and changes.

II THE AVON-HEATHCOTE ESTUARY

Estuaries are very vulnerable to man's intense socio-economic activities (The National Estuarine Pollution Study 1970), and the collection of information on all aspects of their [illegible]

biological, physical and chemical interrelationships is recognised internationally as an urgent priority for research (FAO Fisheries Reports. No.99). The Avon-Heathcote Estuary has a recent history of increasing use for the disposal of domestic and industrial wastes. This situation is typical of estuaries adjacent to urban areas.

This estuary is located within ten miles of the centre of Christchurch, a city with a population of over 125,000 people. The estuary has an area of 8km^2 and roughly forms an equilateral triangle with the Avon River, the Heathcote River, and the mouth to the open coast, conecting with each of the three corners of the triangle respectively (Fig.1.1). The Avon and Heathcote Rivers arise from springs and meander slowly towards the estuary draining a total catchment of 190km^2 comprising suburban and industrial land. Along one side of the triangle, between the Avon and Heathcote Rivers the Christchurch Drainage Board Sewage Works discharge over 28 million gallons of sewage per day into the estuary (Knox and Kilner 1973). After secondary treatment by oxidation this sewage is discharged onto the estuarine mud flats through outfall pipes (Fig.1.1) and dispersed by the ebb tide.

This estuary has a long tradition of broad-based research (Knox and Kilner 1973), which has highlighted a vast array of problem areas for investigation.

III THE STUDY ORGANISM: *Amphibola crenata*

The estuarine mud flat pulmonate gastropod, *Amphibola crenata*, (Martyn 1784), has always been regarded as a curiosity by scientists. One of New Zealand's first nature study text books,

'Nature in New Zealand' compiled by J.Drummond (1902), described it accordingly:

> 'The most interesting shell, from a scientific point of view is the amphibola, which is found in especially large numbers in the brackish waters of the estuary near Sumner, Canterbury. Its peculiarity is that it is an airbreathing snail, which for some reason or other, has gone to live in the saltwater. The amphibola is found in New Zealand alone, and is one of the scientific wonders not only of this country but of the world.'

The estuary specifically mentioned is the Avon-Heathcote Estuary.

This endemic monotypic species probably descended from an original stock which established itself in New Zealand in the Pliocene and which is now also represented by the widespread genus *Salinator*. In early descriptions there was confusion between these two genera of this family: Amphibolidae (Watters 1964). It is one of the few marine pulmonates in Australasia and it has an operculum and a veliger larva which point to archaic relationships (Morton and Miller 1968).

A number of studies, apart from early taxonomic descriptions, have examined different aspects of the species' biology and included observations on its distribution. The early work by Farnie (1919, 1924) provided a very competent framework for subsequent studies by describing its general habits, external features and internal anatomy,and the egg, embryonic and larval development. The main studies on its ecology have been Watters (1964) and Briggs (1972). Watters (1964) described the general distribution of *Amphibola* in Hoopers Inlet on the Otago Peninsula, with reference to sediment size, salinity and exposure.

Briggs (1972) related its general distribution in a population in the Whangateau Harbour, north of Auckland, to feeding and assimilation, and reproduction and growth studies. Kilner (1969) briefly compared the general distributions of *Amphibola crenata* and *Zeliloma subrostrata* in the Avon-Heathcote Estuary. In addition, observations of *Amphibola* habits and distribution have been included in most general surveys in New Zealand of estuarine, upper harbour and mud flat ecology. There is general agreement in all these studies, on the following distribution of *Amphibola*:

(i) it is associated with soft mud to muddy-sand substrate in sheltered bays and upper reaches of estuaries and harbours;

(ii) it is most common above M.T.L., reaching a maximum just below M.W.S., but may occur down to L.W.S.;

(iii) young (or small) individuals are associated with finer sediment material than adults; and

(iv) although its archaic lung predisposes it towards survival in rich organic sediments, *Amphibola* is limited by severe pollution.

Most intertidal animals have an activity or feeding cycle which is related to the period of emersion or submersion, according to their mode of feeding. *Amphibola* breathes air and feeds by passing surface sediment deposits through the gut as it moves over the substrate, digesting flagellates and ciliates as the predominant food item (Jolly 1971). Individual *Amphibola* may be active after the overlying water depth has reached at least 0.5m but generally most bury in the sediment prior to submergence by the flood tide (Watters 1964, observations by the present author). Jolly (1971) and Morton (1975) observed that this activity cycle was modified by season. During winter in the Avon-Heathcote Estuary, *Amphibola* continued to move on the substrate surface after

submergence, although there was some individual variation which seemed to be related to size of the individual and the particular area of the estuary (Jolly 1971). Indirect evidence, however, from changes in stomach contents and digestive diverticula over 24h. indicates that in winter at Barry's Point, Auckland, *Amphibola* is active only during the period of daytime low water, whereas in summer it is active in this area at both low water periods each 24h. and during high water at night (Morton 1975). This apparent contradiction between the conclusions of these two studies may be a result of latitudinal differences, and the limited number and duration of observations in each study. *Amphibola* possibly buries itself in response to the flood tide to avoid unfavourable water conditions, or dislocation by water currents to unfavourable conditions.

Amphibola crenata seemed an appropriate species in which to study the susceptibility of an intertidal estuarine organism to withstand the effects of pollution because:

(i) despite its conspicuous ubiquity in New Zealand estuaries, which are often modified by the disposal of large quantities of domestic and industrial wastes (McLay et al 1975), there have been no studies of the ecology of *Amphibola* in relation to its distribution near to a pollution source;

(ii) although the pulmonate lung allows it to exploit rich organic and often anaerobic conditions without stress from oxygen depletion, population density and size distribution appear to be affected by proximity to sewage outfalls in the Avon-Heathcote Estuary; and

(iii) the restricted mobility of the post-larval snail limits its ability to actively avoid pollution.

IV SOME POSSIBLE INDICATORS OF SUB-LETHAL STRESS

The importance of studying sub-lethal effects of stress from pollution (or artificial modification of the environment) has been emphasised as a necessary progression from acute toxicity studies (e.g. Mitrovic 1973). A species' ability to survive in a particular environment extends from maximum activity, growth, and reproduction in optimal preferred conditions to tolerance, avoidance, and chronic and acute effects resulting in death, in conditions of stress. Under natural conditions, sub-lethal levels of a stress factor may lead to death, depending on intraspecific variability in resistance, effects of season and life stage, interaction with other factors, and interspecific competition. Indeed, sub-lethal changes may have greater long-term implications for the survival of a population than a sudden die-off from acute toxic conditions. Sub-lethal effects have been investigated and identified at all levels of a species' biology. A review by Sprague (1971) cited changes in intracellular structure, biochemistry, physiology, behaviour, reproduction, development, growth, gross morphology and genetic makeup in response to stress from pollutants added to the normal environment of a species.

In the present study, from 1974 to Dec.1976, *Amphibola crenata* collected from each of two areas of the estuary; the western slopes adjacent to the sewage outfalls, and the eastern slopes, were compared with reference to:

(i) shell morphology, based on linear dimensions, weight, geometrical parameters, and patterns of shell deposition at the aperture lip;

(ii) seasonal changes in size frequency distribution described

at monthly intervals and related to the breeding season and larval development (size-frequency distribution in the two study areas was also compared with that in other areas of the estuary);
(iii) reproductive development described by histological examination of gonad follicles at monthly intervals; and
(iv) sediment preference compared by laboratory experiments and description of sediment ingested in the field.

In addition to these studies based on a comparison of areas in the Avon-Heathcote Estuary, the toxicity of some heavy metals (copper and zinc), and the effect of salinity and temperature on the mortality curve, were investigated in the laboratory.

Physical factors such as salinity, temperature, and sediment particle size and organic content were sampled regularly in both study areas for comparison, to identify variables, apart from location with respect to the sewage outfalls, which may affect the biology of *Amphibola*.

CHAPTER II

THE PHYSICAL ENVIRONMENT

I. INTRODUCTION

Salinity, sediment characteristics, exposure time, and dissolved oxygen are considered generally as the main abiotic factors which determine the distributions of resident benthic estuarine organisms (e.g. Tenore 1972). Biotic factors, however, such as inter- and intra-specific competition, and effects of pollution can modify these distribution patterns.

Amphibola crenata has considerable tolerance to both freshwater and seawater (100%) (Farnie 1919, Watters 1964, and this study, Chapter VI), such that Watters (1964) concluded that salinity was not a factor determining size distribution. Salinity can affect the distribution of an animal in a variety of ways, however. For example, the absolute values of the salinity extremes, the rate of change during a tidal cycle, the duration of exposure to salinity or the synergistic action of salinity with another factor, can affect the suitability of a habitat for a species. Salinity change in the surface water prior to tidal submersion may be a stimulus necessary to trigger burrowing in *Amphibola*.

Sediment particles in an estuary are eroded, transported,

resorted and deposited by tidal, river, wave and gravitational energy. In addition to these hydraulic processes, sediment is affected by chemical and biological processes (Postma 1967, Meade 1972). In turn, sediment deposition affects estuarine organisms directly and indirectly depending on the degree of interaction that a species has with the sediment. *Amphibola* is a component of the surface biota or epifauna, and interacts directly with the sediment in its feeding, burrowing and egg laying. Eggs are cemented into a structure of sediment particles. Sediment structure and water content affect the digging rate of a burrowing animal (Chapman 1949), and this may be significant to large *Amphibola* adults, which are not streamlined. Indirectly, sediment structure affects the amount of food present by holding organic material, and as an environmental factor affecting the immediate food organisms of *Amphibola*.

Particle size is usually correlated with organic content because of either a common source of fine grains and organic material, or chemical and physical processes which link the deposition and accumulation patterns of these two factors. Newell (1965) showed that for different benthic studies there was a log relationship between percentage organic carbon and median grain size which depended on the fluidity of the mud. Percentage by weight of organic material in sediment is usually small but studies by Marshall (1972) and Johnson (1974) showed that the small organic component comprised a great diversity of potential food particles including organic-mineral aggregates, faecal matter, plant fragments, micro-algae, and other items, which all provide a vast substrate for micro organisms. This component may have physical and biological effects which are

much more important to the biological community than its percentage presence in the sediment would suggest. The proportion of silt and clay present affects the binding of organic material, and together these inorganic and organic components build up a matrix which affects further silt and clay deposition, the flux of organic materials and the adsorption and binding of pollutants such as heavy metals (Toth and Ott 1970).

Temperature was measured as an indicator of seasonal changes during the study period. Dissolved oxygen was not measured in this description of the physical environment as it was considered to be probably unimportant to a pulmonate such as *Amphibola*.

II METHODS

(1) Sampling Sites

Stations 1 and 2 (Fig.2.2) on the western slopes of the estuary were located 50m and 200m, respectively, from the main sewage outfall, and at a tidal level of approximately 2.5h. below H.W.L. These two stations were selected to represent the area populated by a sparse distribution of adults, and dense occurrence of juveniles, on the upper intertidal slopes adjacent to the sewage outfalls (Chapter 1). Stations 3 and 4 were located on the eastern slopes of the estuary at the same tidal exposure level (2 to 2.5h. below H.W.L.) and a similar salinity profile (Fig.2.2) as at stations 1 and 2.

Stations 1 and 2, and stations 3 and 4 therefore provided replicate sites within two areas of comparable salinity, exposure, and location with respect to the Avon River, and distance from the sea. These two areas were believed to differ predominately in

relation to distance from the sewage outfalls. Each station was defined as a rectangle 20m long parallel to the tidal level, and 5m long normal to the shore, and identified with permanent wooden stakes. On any sampling occasion two replicates were randomly chosen within each rectangle.

Stations 1, 2 and 3 were subject to unchannelled freshwater surface drainage from aquifers (stations 1 and 2) and discharge from a drain (station 3). Bathymorphic profiles (Fig.2.1) and height above datum contours (Macpherson 1977, Map 1) show that there was a relatively high angle of slope at stations 1 and 2, and a low angle at stations 3 and 4.

Although the rectangle was of the same area at all four stations, the dimension normal to the shore at stations 1 and 2, with a higher angle of slope covered a wider exposure range than at stations 3 and 4.

(2) Salinity

Salinity was sampled on two different occasions.

(i) Stations 1 and 2 (sites 1 to 4), and sites 5 to 17 along the eastern slopes, May 1974.

To select sites for stations 3 and 4 with the same salinity profile as that at stations 1 and 2, the salinity range at two sites at each of stations 1 and 2 was compared with a sequence of 13 sites (numbered 5 to 17, Fig.2.2) along the eastern slopes of the estuary. Over a week at the beginning of May 1974 salinity was measured at 30 min intervals (Fig.2.4) on the flood tide at sites 1 to 17, at approximately the same tidal level (H.W. occurred between the samples taken 105 min and 135 min after submergence). Water samples were taken by digging a shallow hole in the sediment and collecting the mixture of surface and

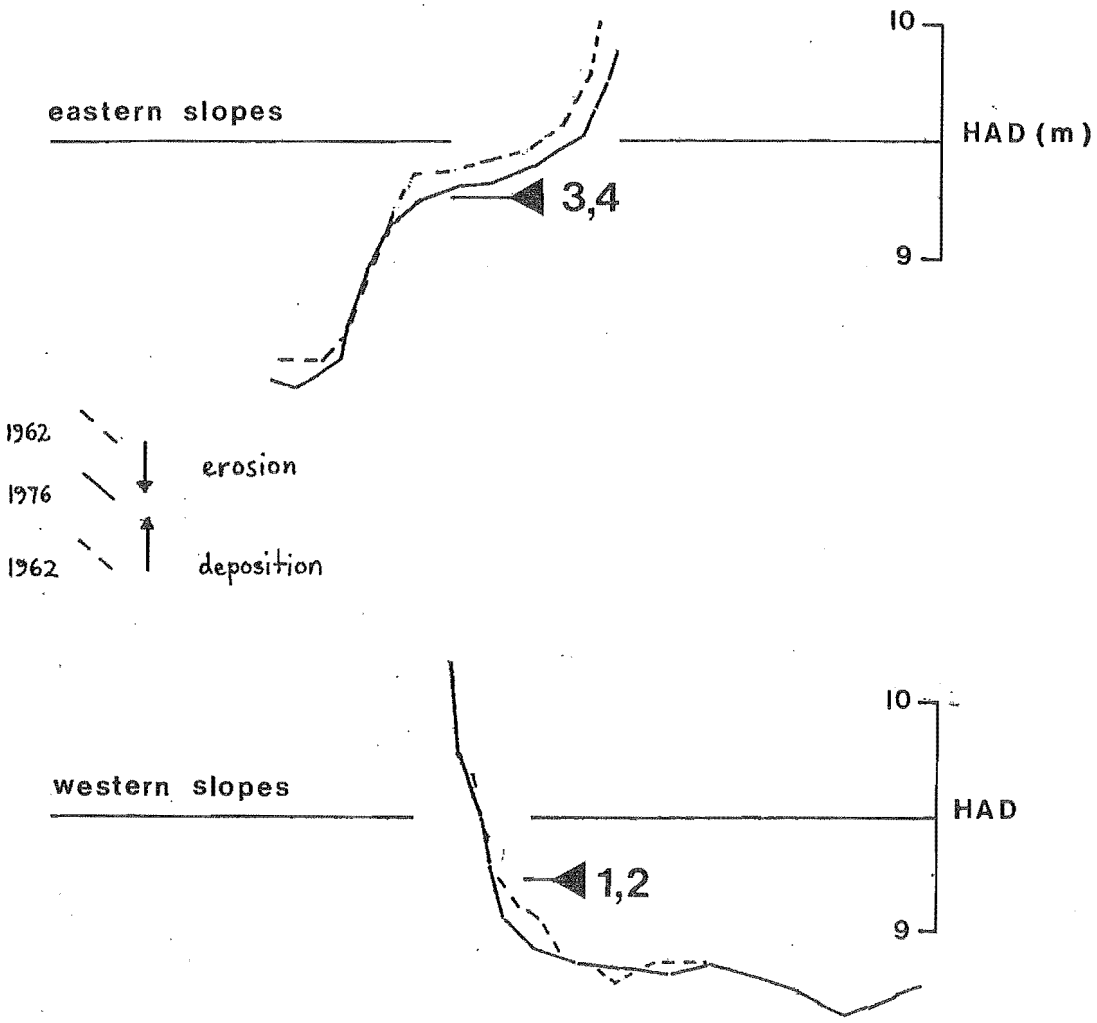


Fig.2.1. Bathymorphic profiles normal to the shore, looking north for the eastern slopes and western slopes, after Macpherson (1977: profile 5, Fig.23, p.53, and profile 7, Fig.28, p.60). Stations 1 and 2, and stations 3 and 4 are indicated at about 9.25 HAD (Height above datum). Profiles surveyed in 1962 and 1975, 1976 are compared.

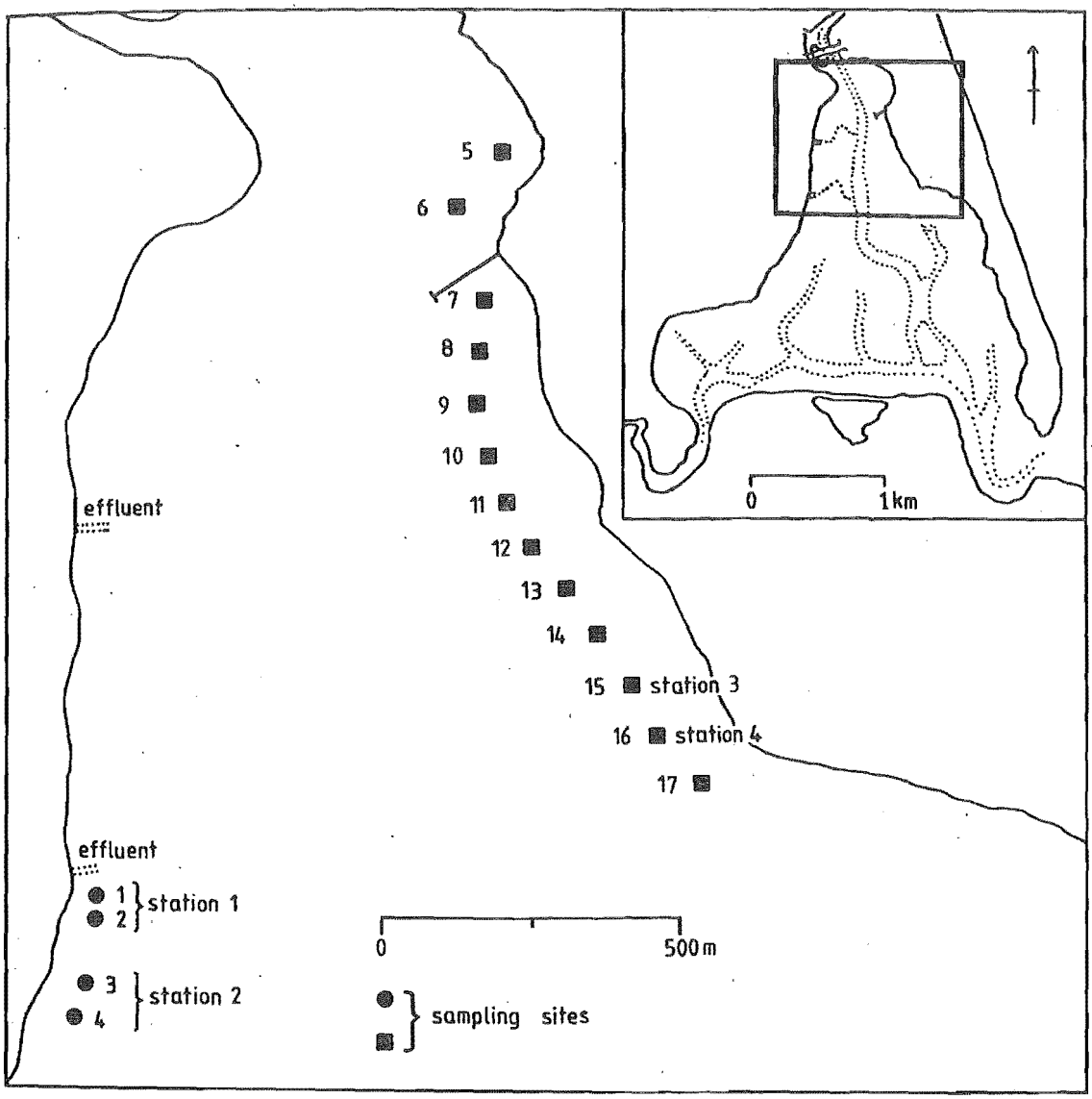


Fig.2.2. North segment of the Avon-Heathcote Estuary showing sites 1 to 4 on the western slopes, and sites 5 to 17 on the eastern slopes. Salinity was measured at each of these sites and stations 1,2,3, and 4 were subsequently located as shown. The effluent outfalls from the Christchurch Drainage Board sewage ponds are also shown.

interstitial water which drained into the depression. This mixture was assumed to be representative of that encountered by *Amphibola* during its emersed period of surface feeding and burying.

After submersion of the sampling site water samples were collected from the bottom of the water column by lowering a plastic container down close to the sediment surface, removing the lid to fill the container with water and replacing the lid before raising the container from the sediment surface. All samples were collected in 250 ml plastic containers and removed to the laboratory where conductivity was measured the following day on an inductively coupled salinometer (Autolab Industries, Model 601, Mark III), against standardised seawater, and converted to salinity ($^{o}/oo$) by reference to a conversion table.

(ii) Comparison of stations 1 to 4, Sep. 1974 to Feb. 1977 (Fig.2.2).

In May 1977, water samples were collected hourly over a 12h. period, at two sites at each of stations 1 to 4, using the techniques described above. These samples provided a description of changes in salinity over a full tidal cycle.

(3) Sediment particle size

Sediment samples were taken at stations 1 to 4 in Sep. 1974; Jul. and Oct. 1975; Jan., May, Sep., and Dec. 1976; and Feb. 1977. Sediment was also collected at H.W.L. above station 3 in Dec. 1976. During the survey of *Amphibola* in 1975 from throughout the estuary (Chapter IV), sediment samples were also taken at different tidal levels along transects 1H to 6H (Heathcote River) and 7A to 9A (Avon River)(Fig.2.3). These provided sediment information for areas within the range of

Amphibola in the lower Heathcote and Avon Rivers but outside of the area described by Macpherson (1977).

Sediment samples comprised 100g of surface material scooped from the top 10mm of sediment. Wet sieving was used for the initial separation of the sand (greater than 0.0625mm or 4ϕ) from silt and clay. Dry sieving separated the different sand size classes, and pipette analysis was used for the silt-clay fractions. The methods and precautions described by Folk (1968, p 34-38) were closely followed using standard equipment in the Department of Geology Sedimentation Laboratory, University of Canterbury. Wet sediment samples were pretreated with hydrogen peroxide (30%) to digest organic material and breakup organic-mineral aggregates, and sodium hexametaphosphate (Calgon) was used as a dispersant in the pipette analysis.

(4) Organic content

Both organic carbon and organic nitrogen can be used to provide measures of the amount and source of the organic material present. Organic carbon is determined by dry combustion weight loss, or hot or cold wet chemical digestion. All of the methods have limited reliability for variable estuarine muds (Morgans 1956; Wakeel and Riley 1956): ashing because of the loss of bound water in the clays, and carbonates from shell debris; and wet oxidation because of the presence of extraneous reducing substances. To achieve a simple comparison of relative values of organic carbon in surface sediment at each station in this study, weight loss of small sediment samples was measured after dry combustion in a furnace. This was found to have acceptable reproducibility if shell fragments were carefully removed before analysis.

Sediment samples were collected at stations 1 to 4 in Sep. and Oct. 1975; Jan., May, Sep., and Dec. 1976; and Jun. 1977, and along a transect from H.W.L. to L.W.L. up the shore at station 1, and along transect 9A (Fig.2.3) in Jun. 1977. Samples of 10ml of surface sediment were scooped from the top 5mm of substrate and, after mixing, subsampled for 3g wet weight of sediment. This was spread as thinly as possible around the inside of a preweighed crucible and dried overnight at 60°C. After being weighed, the sample was ashed to constant weight (2h. at 450°C) in a closed furnace, and reweighed. After drying, and ashing, the sample was cooled in a desiccator to avoid moisture uptake before weighing.

(5) Temperature

Air and mud temperatures at stations 1 and 3 were measured as part of the regular monthly sampling programme for *Amphibola*. Fine calm days were usually chosen for sampling which led to a bias in the temperature pattern. Air temperature was measured by holding a dry thermometer 10cm above the sediment surface in the direct sun, and mud temperature was measured at 1cm (surface) and 5cm below the sediment surface.

III RESULTS

(1) Salinity

(i) Stations 1 and 2 (sites 1 to 4) and sites 5 to 17 along the eastern slopes, May 1974.

At stations 1 and 2, prior to submergence, salinity was relatively constant at 5 to 10°/oo but ranged up to over 25°/oo where fresh water was not flowing over the sediment surface

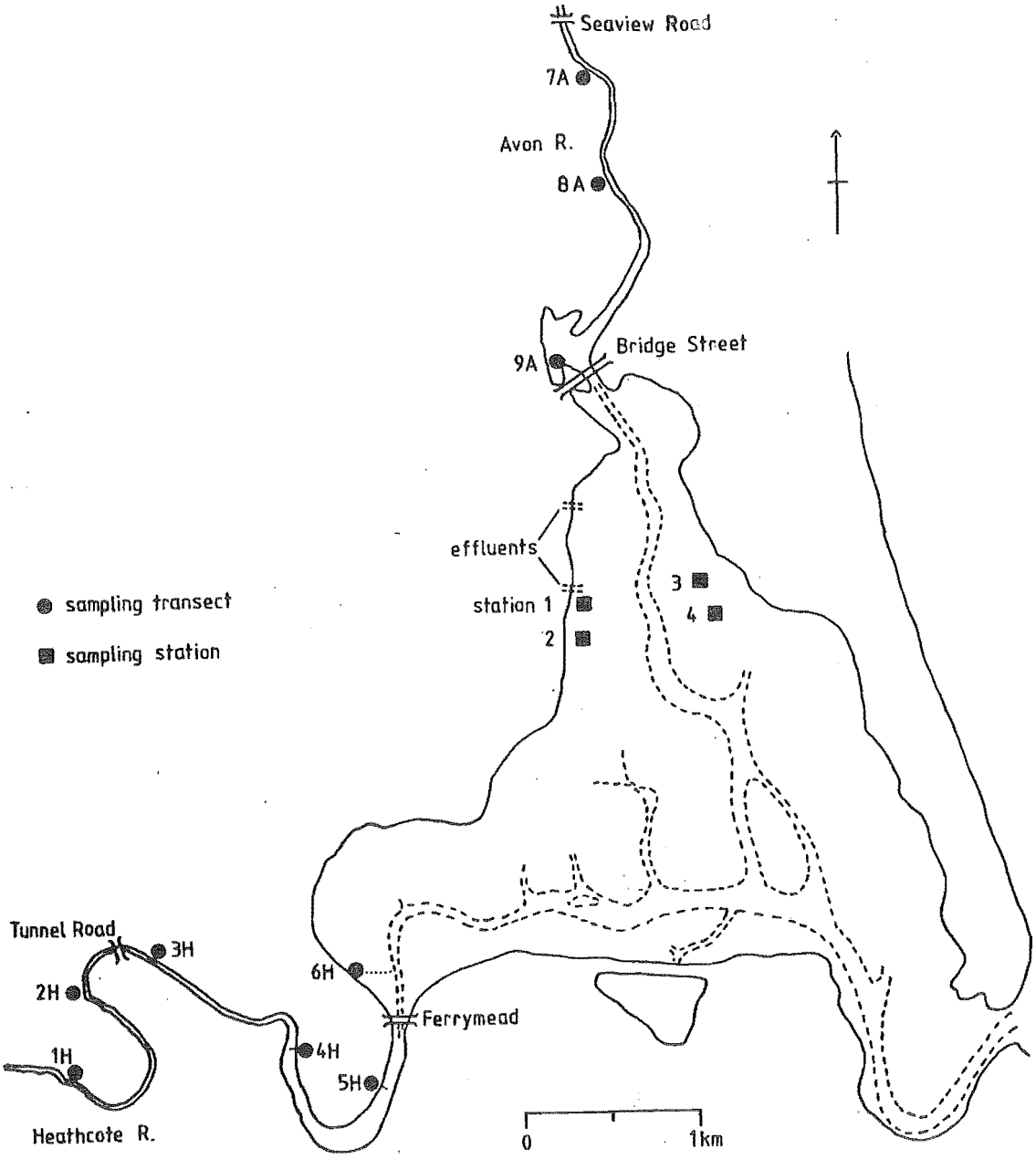


Fig. 2.3. Lower reaches of Heathcote and Avon Rivers and Avon-Heathcote Estuary showing locations of transects 1H to 6H and 7A to 9A, sampled at different tidal levels for sediment particle size analysis and *A crenata* size distribution.

(Fig.2.4). Salinity increased rapidly at stations 1 and 2 during the first 15 min after submersion, more slowly over the next 30 min and then increased rapidly to a maximum of 30°/oo at H.W. The trend along the eastern slopes towards the mouth of the estuary was an increase in salinity for any given time after submersion, but the magnitude of change between submersion and highwater was relatively constant at about 20% for all sites. A relatively abrupt increase in salinity occurred for all times after submersion at about the South Brighton jetty where the estuary narrows into the Avon River, and soon after submersion, between sites 14 and 15. South of site 15 the salinity profile reached a plateau which closely approximated the profile on the western slopes at station 1 and 2, although the rate of salinity change was greater immediately after submersion on the eastern slopes.

The effect of wind was to lower salinities by up to 10% as shown by sites 7, 8 and 10 which were sampled on both a calm day, and under turbulent conditions caused by a strong southwest wind.

(ii) Comparison of stations 1 to 4, May 1977.

Stations 1 to 3 generally had lower salinities and a wider range (5 - 25°/oo) before submersion than station 4 (26°/oo) (Fig.2.5). At the time of submersion the salinity first dropped at each station before rising rapidly to a maximum of about 32°/oo at about the time of highwater, although there appeared to be a lag at station 2. Salinity then dropped rapidly with the ebb tide to about 25°/oo at the time of emersion. This level was relatively constant at first, and then varied more within each station as the effect of freshwater surface flow was expressed.

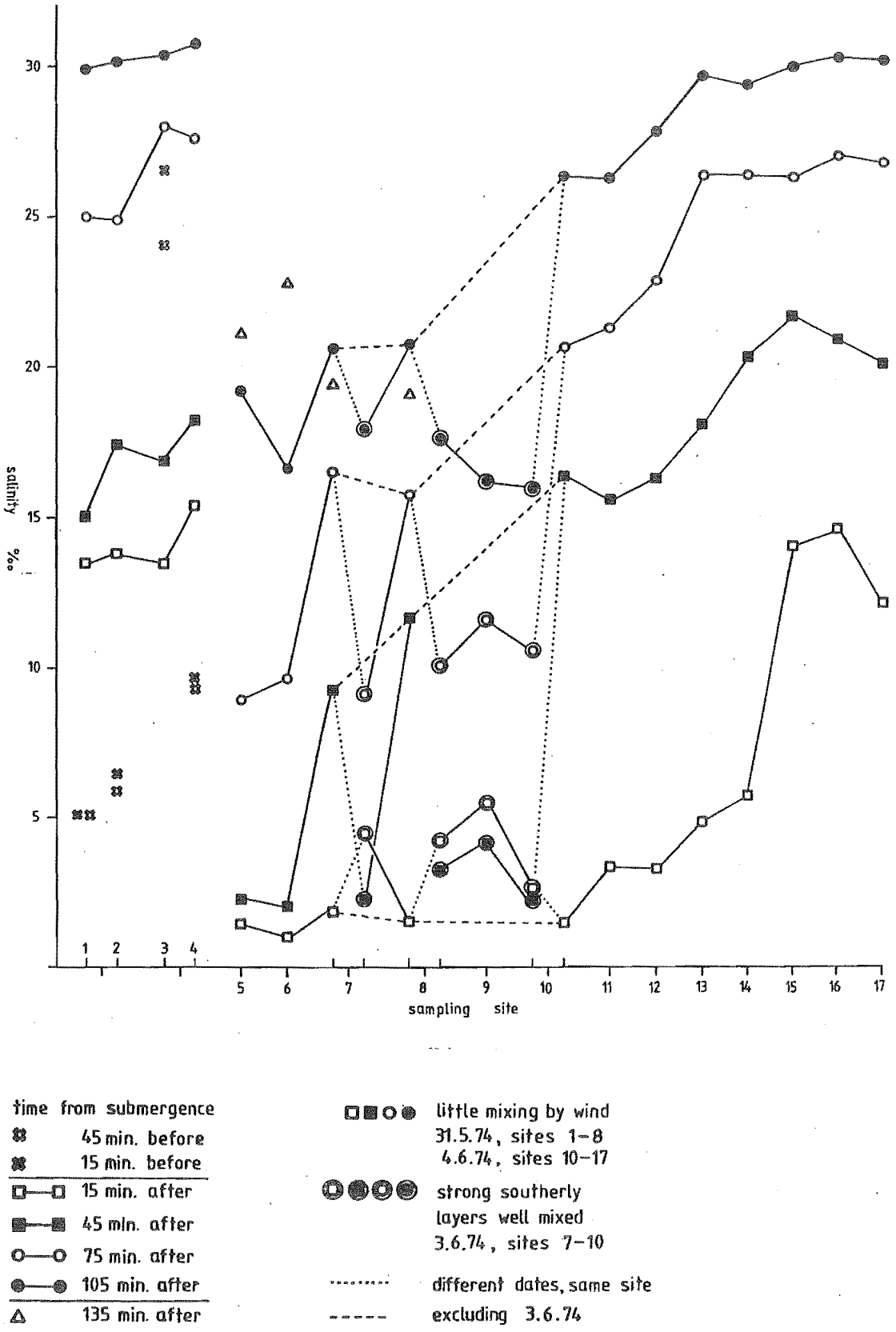


Fig. 2.4. Salinity (0/00) at sites 1 to 17 shown on map (Fig. 1.1.). Each line connects points describing salinity at the same time with respect to submergence at different sampling sites. Sites 7, 8 and 10 were sampled on two different days under different weather conditions.

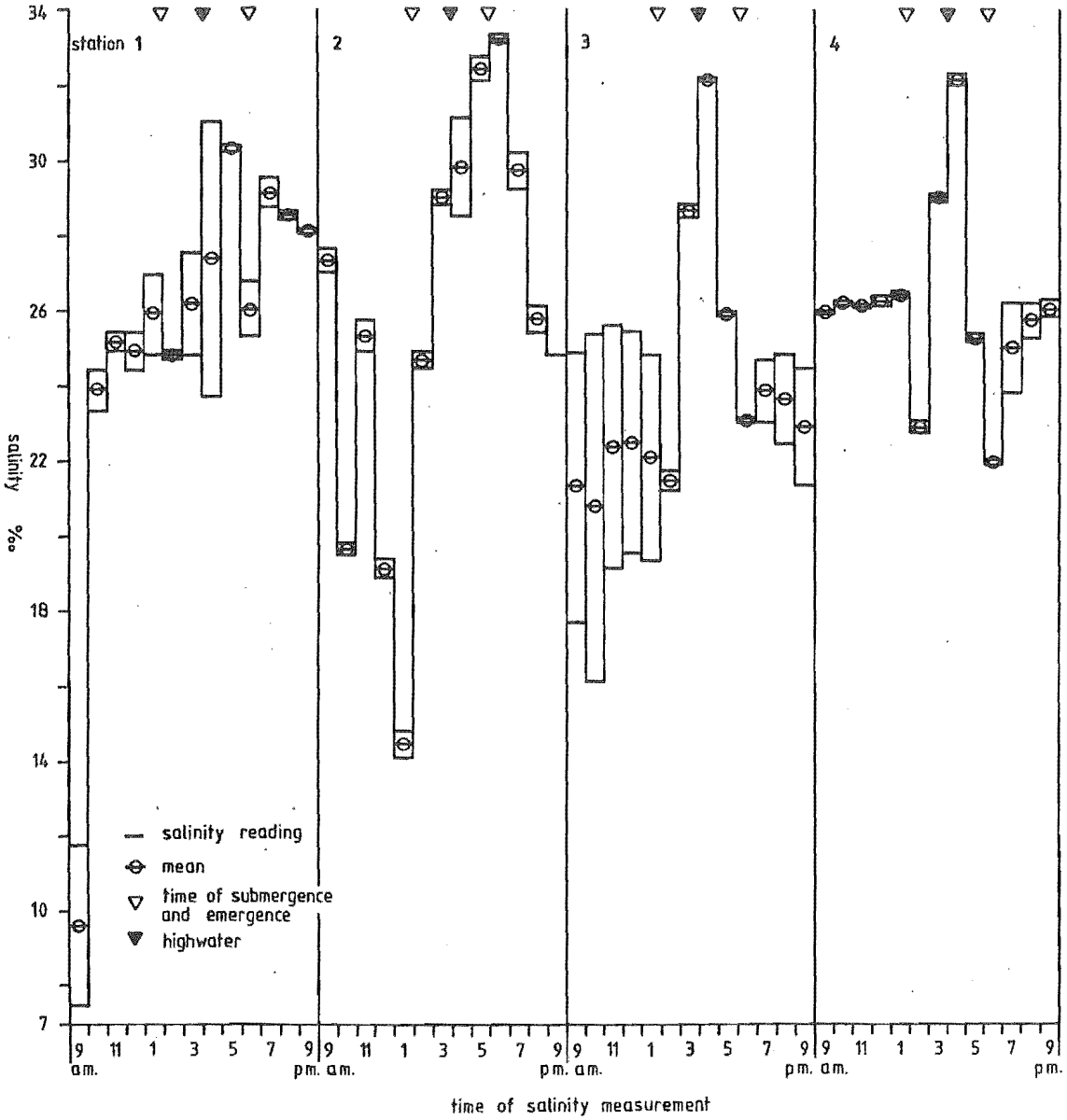


Fig. 2.5. Salinity (0/00) at stations 1 to 4 measured over 12h and including one full submersion period. Two replicate measurements and the mean are shown for each sampling event.

(2) Sediment Particle Size

Sediment grain size classes generally follow a log-normal distribution and various statistics have been suggested to describe the curve generated by plotting cumulative percent of sample weight against $\phi(-\log_2$ grain diameter in mm) (Folk 1966; Friedman 1967). Morgans (1956) discussed the importance of using such statistics in ecological work. The histogram cannot be used directly to calculate these statistics but it provides a simple and pictorial means of comparing different sample. Klovan (1966) in fact, advocated the use of the percent weight of sediment in each class interval for computing statistics which utilise more information and are more meaningful for describing depositional environments. In this study, the results of sediment analyses at stations 1 to 4 during the study period are presented as histograms.

(i) Comparison of stations 1 to 4, Sep. 1974 to Feb. 1977.

The two replicates at each station on each sampling occasion were consistent with each other, and means of the replicates were used to compare stations (Fig.2.6). The pattern of different grain size classes was similar at stations 1 and 2, and at stations 3 and 4. The grain size pattern was also relatively constant throughout the period from 1974 to 1977.

Stations 3 and 4 had about four times more coarse to medium sand and shell fragments (2.0 ϕ) than stations 1 and 2, but the amount at all stations was less than 40%. Medium-fine sand (2.0 to 2.5ϕ) was in similar proportions (10 to 20%) at all stations. The predominant size class at all stations (stations 1 and 2, about 50%; stations 3 and 4, about 60%) was fine sand (2.5 to 3.0ϕ). The mode, median and graphic mean (Folk 1968) lay within the limits of this size class. Throughout most of the period

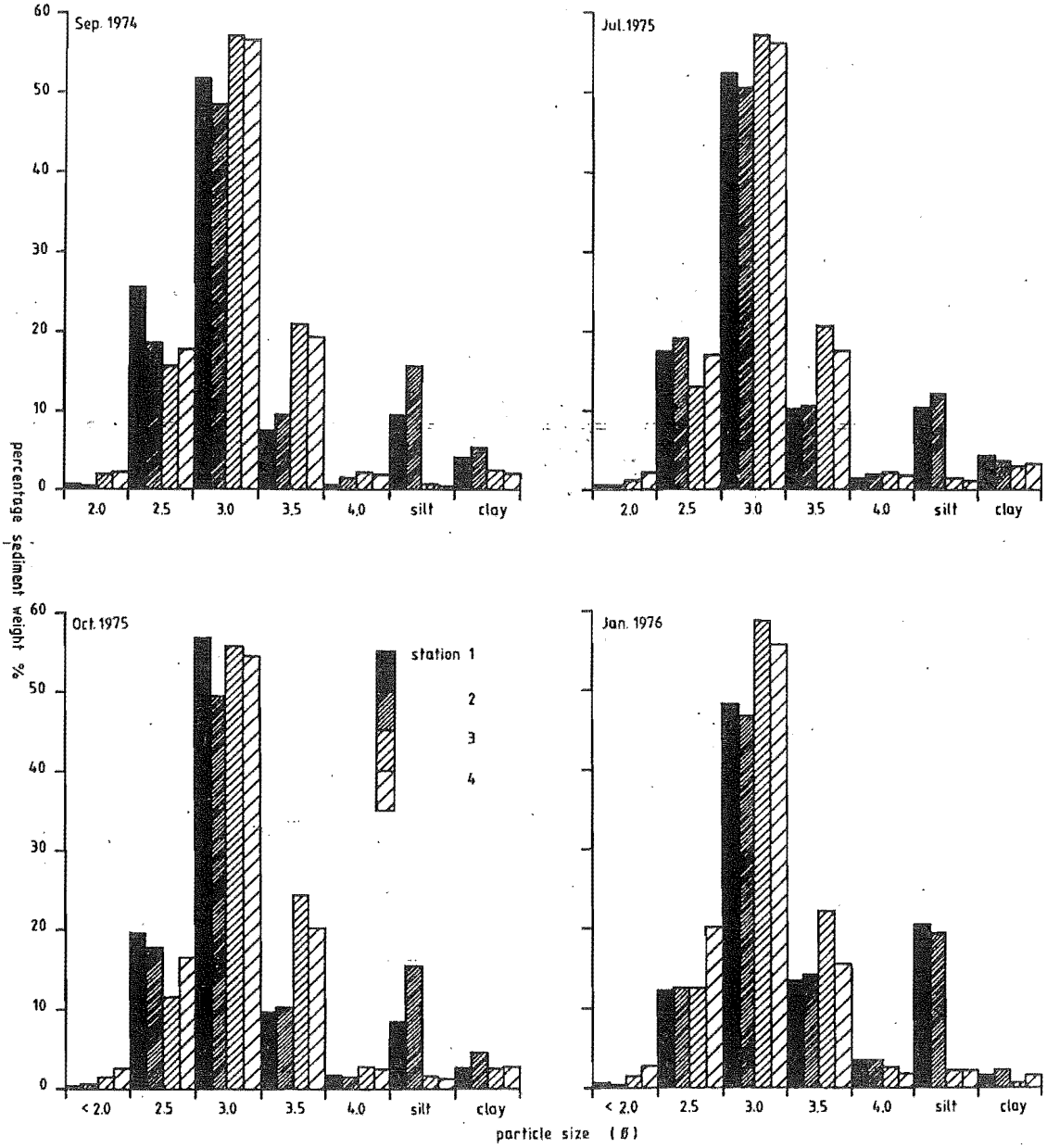


Fig. 2.6. Percentage sediment weight of each particle size (ø) for each station (1 to 4, and above station 3 in Dec. 1976), from Sep. 1974 to Feb. 1977).

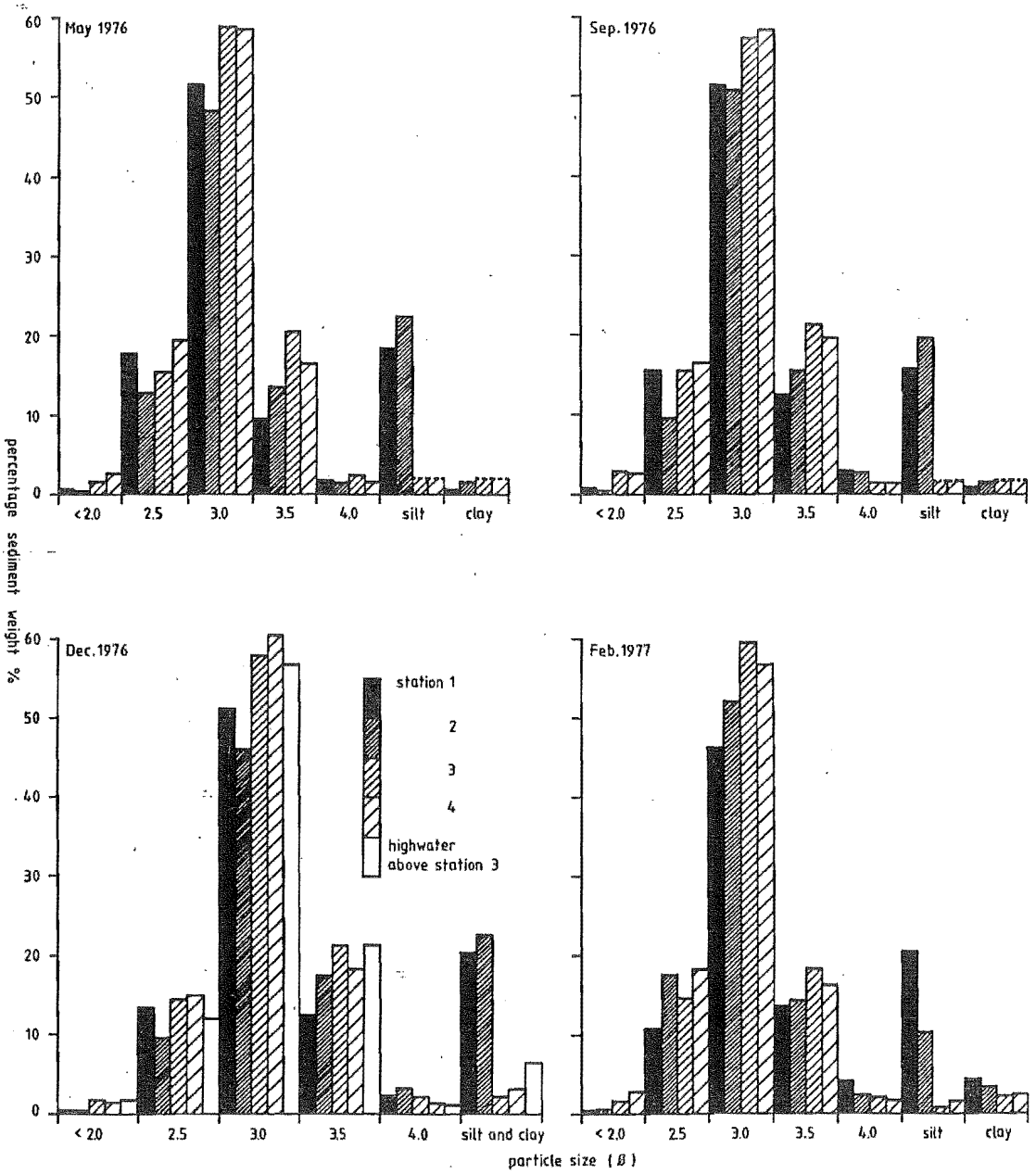


Fig. 2.6. Continued.

stations 3 and 4 had twice the percentage (about 20%) of fine to very fine sand (3.0 to 3.5ø) as that at stations 1 and 2 (10%), although in early 1977 the four stations were approximately equivalent. Very fine sand was present in approximately equivalent proportions (less than 4%) at all stations. The percentage silt (4.0 to 8.0ø) in the individual half-ø classes was not measured and the apparent second mode at this point on the size class axis was an artifact of the contraction of six half-ø classes. The contracted presentation clearly shows a marked difference in silt content between stations 1 and 2 (about 20%) and 3 and 4 (less than 3%). All stations showed a similar percentage (1 to 4%) clay content throughout the study. Most of the difference between the two areas (stations 1 and 2, and stations 3 and 4) was in the silt fraction, which was balanced by the difference in the percentages of fine and very fine sand (2.5 to 3.5ø). The samples taken at H.W.L. above station 3 were similar in composition to station 3, although there was a two fold increase in silt plus clay content, to 6.6%. At this level *Amphibola crenata* size distribution was quite different from that at station 3 (chapter 4).

(ii) Sediment size distribution along transects on the lower Heathcote and Avon Rivers, May 1975.

The Heathcote River transect levels, had a dominant silt fraction (Fig. 2.7). For these transects the sand fractions were combined for easy comparison with the silt and clay fractions. Transects 1H to 4H, and 7A and 8A were normal to the shore up quite steep banks and the individual sampling points along these transects were spatially close, but equivalent to the low slope transects at 5H, 6H and 9A in tidal exposure time. From transect 1H to 5H the predominant fraction was generally silt, which com-

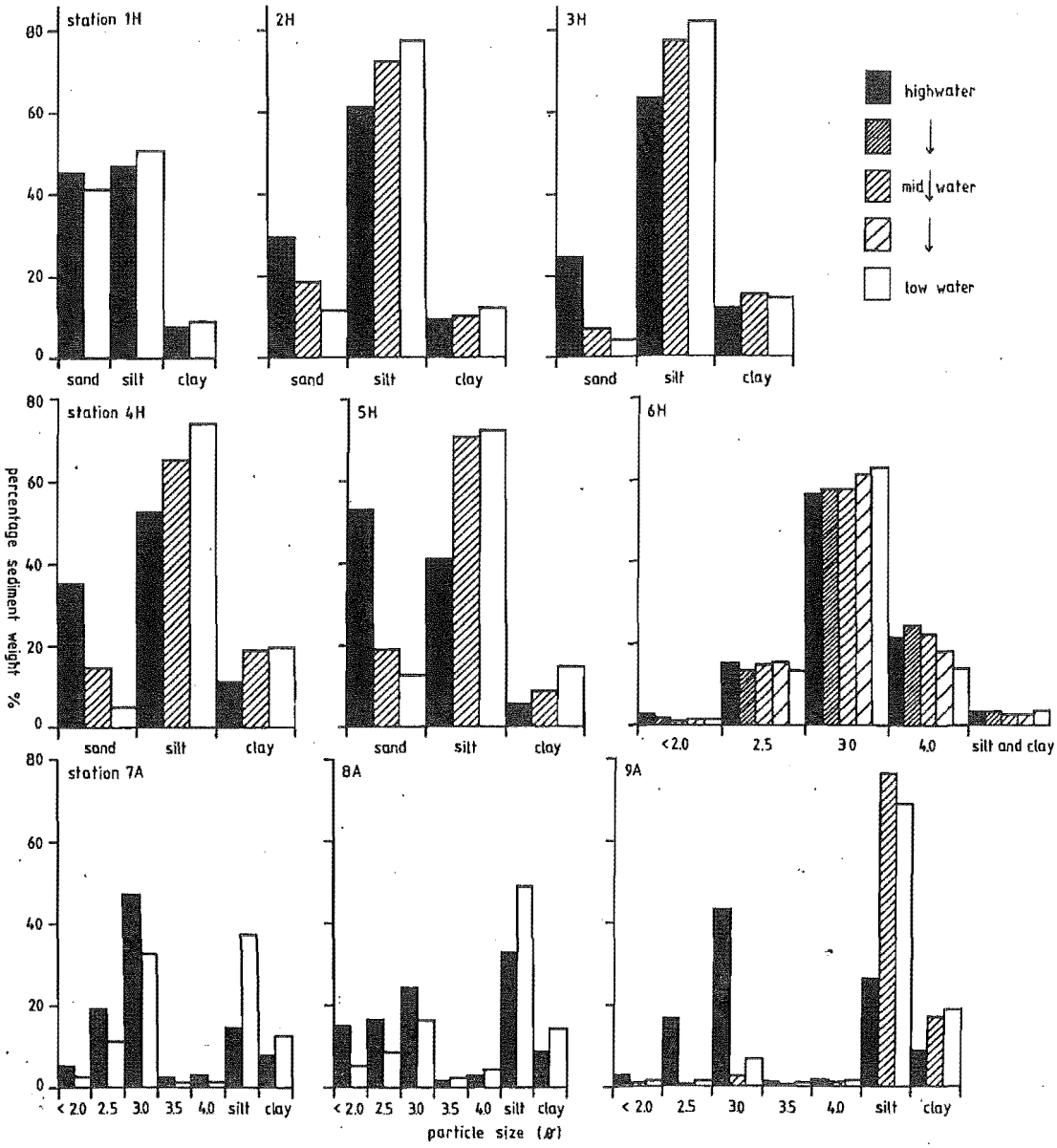


Fig. 2.7. Percentage sediment weight of each particle size category, or ø class, along transects 1H to 6H and 7A to 9A (Fig. 2.3.) at different tidal levels.

prised up to 80% of the sediment, but at H.W.L. at 5H sand exceeded silt content. Sand and clay fractions were almost equivalent in weight, with clay content increasing towards L.W.L. From H.W.L. to L.W.L. at transects 1H to 5H, sand decreased and silt correspondingly increased. A relatively abrupt change occurred below the Ferrymead Bridge where the mud flats spread out. At 6H the sediment pattern closely resembled that of stations 3 and 4 where silt and clay comprised a relatively small proportion of the total weight, and fine sand (2.5 to 3.0ϕ) was the dominant fraction (up to 60%). There was very little change in the percentages of each size class with tidal level at 6H. The predominant sand component at 7A, 8A and 9A in the lower Avon River was 2.5 to 3.0ϕ. With progression down the river along the stretch covered by these transects, the percentage sand dropped for any tide level until silt became the modal class below 3A at L.W.L. and M.W.L., but sand remained dominant at 9A H.W.L.

In both lower river reaches, the grain size pattern with tidal level was consistent along each transect with the highest proportion of silt at L.W.L. but in the Avon River silt did not predominate over sand as in the Heathcote, except on the mud flats above 9A where the river configuration and rush beds create a silt trap. Clay content never exceeded silt content and was always less than 20% of the total sediment weight.

3. Organic Content

Percentage carbon in sediment showed no overall trends and maximum values at one station were not clearly related to maxima at other stations (Fig. 2.8). Organic content at stations 1 and 2 (1.7 to 2.5%) was approximately twice that of stations 3 and 4 (1.2 to 1.6%). This ratio was relatively constant throughout the

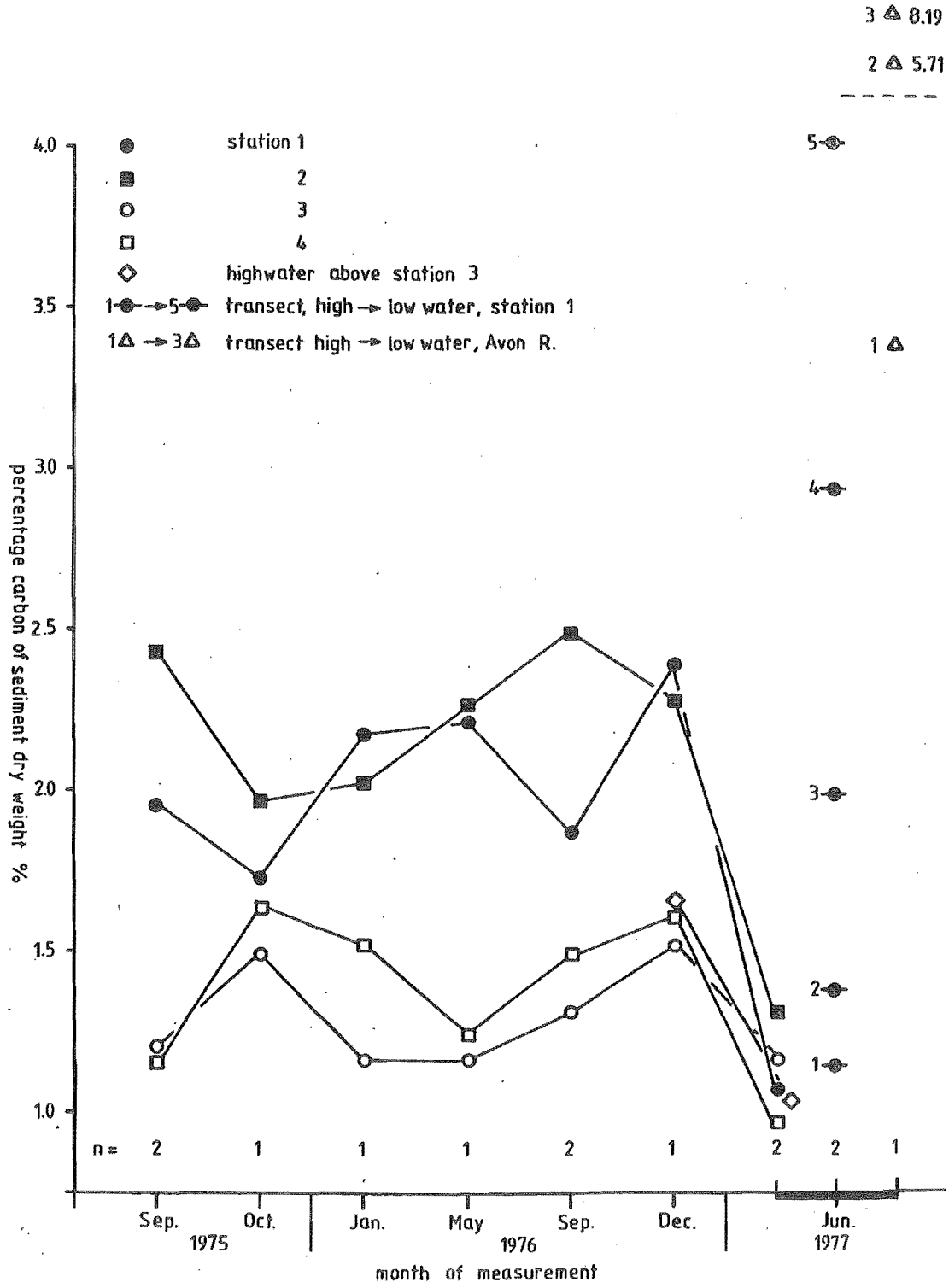


Fig.2.8. Percentage organic carbon of sediment dry weight in surface sediment at stations 1 to 4 from Sep.1975 to Jun.1977, and along transects at station 1, and 9A in Jun.1977.

sampling period except in June 1976, when the level at station 1 and 2 was much lower, but this may indicate a sampling error produced by the patchy nature of these two stations where upwelling surface water can scour fine particles and organic material away. A transect at station 1 from H.W.L. to L.W.L. showed that organic content increased to over twice the station 1 level (site 2 on the transect from H.W.L. to L.W.L.) or to approximately 4% where silt-clay levels were highest, below M.W.L. These levels were compared with those along a transect normal to the shore, along the lower reaches of the Avon River close to sediment transect 9A (Fig. 2.3). Percentage carbon was over 8% in this area of high silt and clay composition at M.W.L. and L.W.L. At H.W.L. where fine sand (2.5 to 3.0ø) was the dominant fraction, the organic content was higher than at stations 1 and 2, although slightly less than at L.W.L. in front of the sewage outfall.

4. Temperature

Temperatures measured over 1974, 1975 and 1976 were combined into monthly means to compare season changes in air and sediment temperatures at stations 1 and 3 (Fig. 2.9). Temperatures ranged from 20 to 25° in Jan. and Dec. (summer) to 7 to 10° in Jun. and Jul. (winter), and were all close to the mean daily maximum for each month which reflects the bias in sampling for fine warm days. During Jan., Feb., Sep., Oct., Nov., Dec. the highest temperatures were recorded in the surface sediment at station 3, followed by the surface sediment at station 1 which was 1 to 2°C less. Surface sediment temperatures were about 4°C higher than the corresponding air temperatures and were usually higher than deeper sediment temperatures during this period. In May, Jun. and Jul. the relationship was inverted when air temperatures were

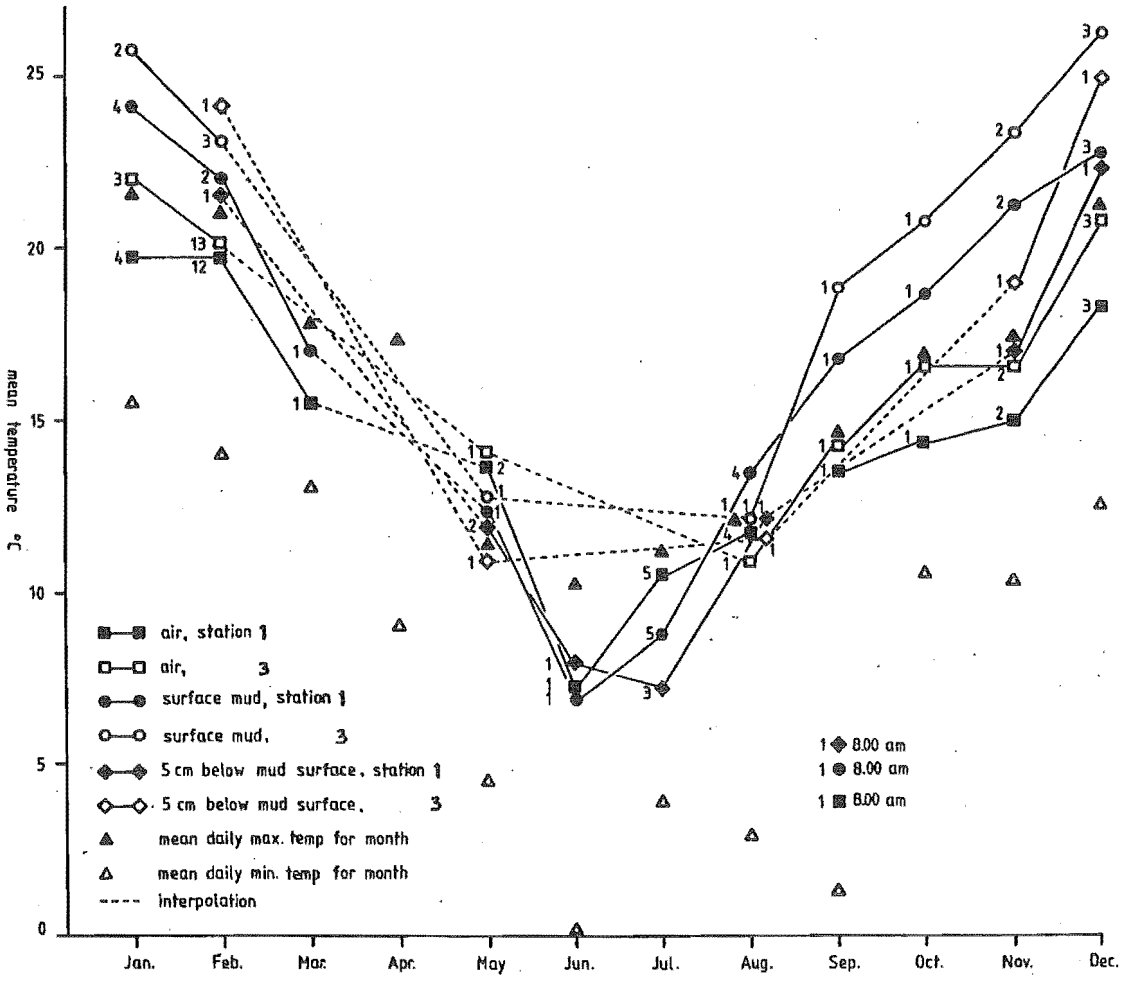


Fig.2.9. Mean air, surface and below surface sediment temperature (°C) for each month, measured during the period 1974 to 1975. Mean daily maximum and minimum temperatures are also shown for each month during this period, as provided by the New Zealand Meteorological Service for the Bromley weather station (H32573), Christchurch.

about 1°C higher than surface sediment temperatures. A full temperature record was not available for station 3 during the winter months.

The lowest temperatures were recorded at 8.00 am during Sep. (4.6°C) when deep mud was nearly 1° higher than surface sediment, which was 1°. higher than air temperature. Temperatures recorded in air and surface sediment at stations 1 and 3 on the same day were also compared (Table 2.1). On one occasion, 16 Feb. 1977, the sediment temperature reached a maximum at station 1 which exceeded that at station 3, but on all other days recorded during summer, station 3 had the highest air and sediment temperature by 1 to .2°C, with sediment temperatures exceeding those in air by 3 - 6°C. During Feb. 1975 when temperature was recorded regularly during an *A. crenata* egg laying study, the range of surface sediment temperatures was the same as the range in air. During May and August, temperatures were higher at station 1 than at station 3. Air temperature was the same at different shore levels at the same time on the day of measurement, but surface sediment temperature at H.T.L. reached greater extremes because of the longer duration that it was exposed to high insolation or low air temperatures.

During May, Sep. and Oct. 1974, sediment and air temperatures at stations 1 and 3 were recorded regularly throughout the day (Fig. 2.10.). Sediment temperatures in May were lower and less erratic than the air temperatures and reached a plateau before dropping to the water temperature level after submergence. A cool bottom layer of water gradually mixed with a warmer surface layer to produce a homogeneous column of water during ebb tide,

Table 2.1 Temperatures °C of air and surface sediment.

Date	Year	Air		Sediment	
		Station 1	Station 3	Station 1	Station 3
13 Jan	75	22.0	23.5	27.5	27.0
24 Jan	75	20.5	20.5	24.0	24.5
9 Feb	75	21.0	20.7	26.5	28.2
		21.0	20.7 (H.T.L.)	27.0	28.8 (H.T.L.)
		21.4	20.7 (M.T.L.)	24.5	25.8 (M.T.L.)
12 Feb	75	20.0	21.5	27.0	28.5
13 Feb	75	21.0	22.0	26.5	28.0
14 Feb	75	20.0	21.5	21.5	22.5
15 Feb	75	18.5	20.0	21.5	25.0
16 Feb	75	20.0	20.0	20.0	22.0
16 Feb	77	21.0	24.2	28.0	26.7
17 Feb	75	16.0	16.0	17.0	18.0
18 Feb	75	18.0	19.0	19.0	21.5
19 Feb	75	20.0	19.5	21.5	22.0
20 Feb	75	22.0	23.0	22.0	23.0
24 Feb	75	20.5	20.0	20.0	20.0
1 May	76	12.0	11.8	12.0	11.8
15 May	77	15.3	14.1	13.0	12.8
7 Aug	76	13.8	11.0	17.0	12.0
18 Sep	74	13.5	14.3	16.8	18.8
5 Oct	74	14.3	16.5	19.6	20.7
19 Nov	76	12.0	13.0	14.0	17.9
20 Nov	74	18.0	19.0	24.0	26.0
26 Dec	76	20.5	22.0	26.5	27.0

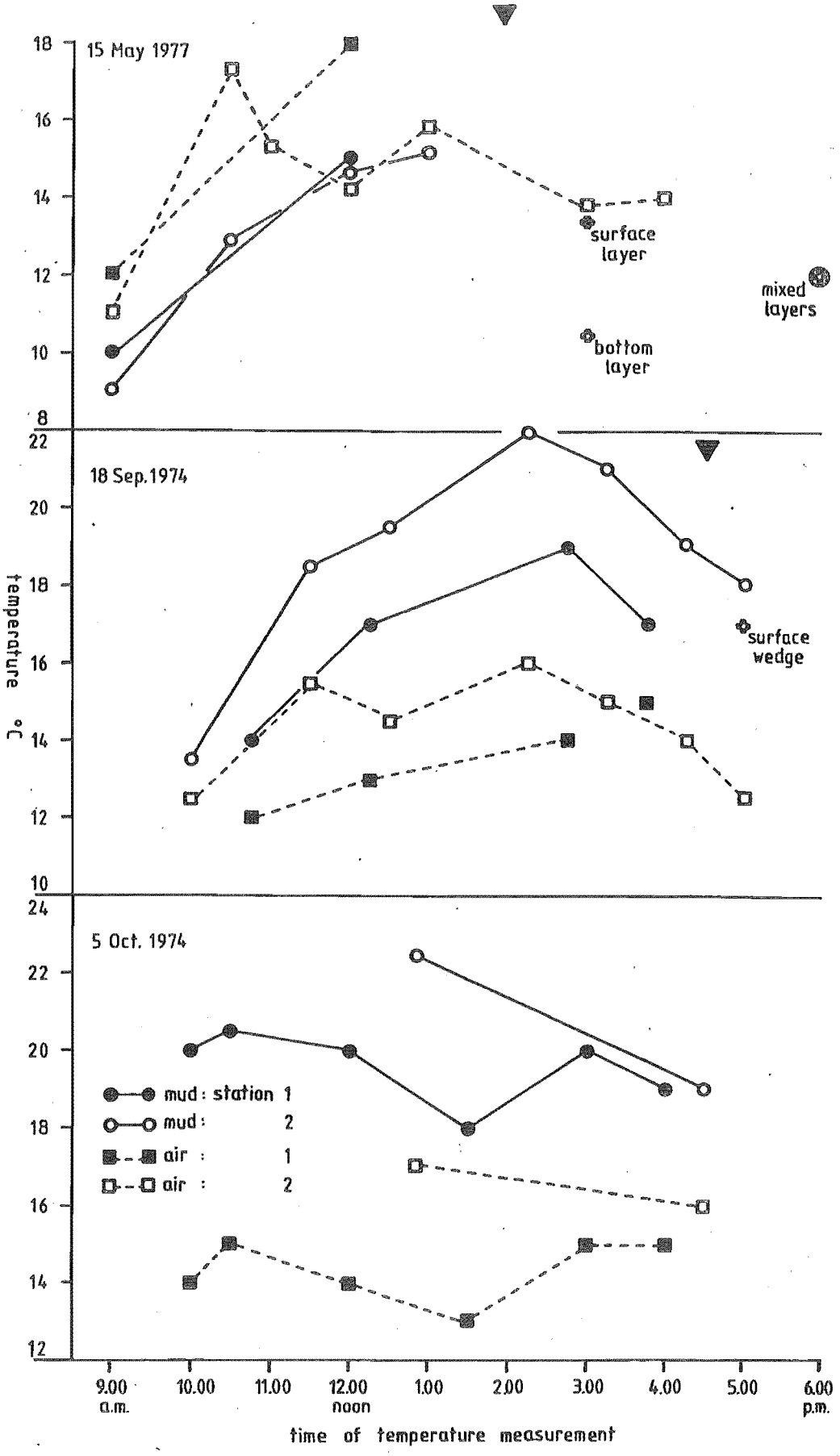


Fig.2.10. Air and surface sediment temperature (°C) at different times during the day at different stations (1 to 4) for May, Sep. and Oct.1974. Water temperatures are shown for measurements taken after submergence of the sampling site by the flood tide. The time of submergence is shown by the large black inverted triangle.

which had a temperature approximately half way between the two initial water temperatures.

In Sep. the sediment temperature continued to rise to a maximum at least 6°C higher than the air temperature which reached a plateau at mid-morning. The air and sediment temperatures dropped at the same rate in the afternoon, with no detectable lag in sediment response to changes in air temperature. This response of sediment temperature to changes in air temperature was also found in Oct. Stations 1 and 3 showed different sediment and air temperatures but the relationship between sediment and air temperatures was consistent in each area.

IV DISCUSSION

1. Salinity

Variation in water salinity during intertidal emersion at each station depended on the amount of freshwater seepage. At stations 1 and 2 freshwater flows through the sand from the sewage ponds about 100m away. After submersion of each station the salinity pattern was consistent with a model of the flood tide comprising a preceding surface layer of low salinity river and mud flat water, followed by a mixed layer graded into the wedge of sea water moving up the estuary from the mouth. The ebb tide appeared to present a reverse of the flood tide sequence.

Direction of floodtide flow shown by Webb (1973) is normal to the shore at stations 1 and 2, and parallel to the shore at stations 3 and 4, but in each case the water comes from the same source in the area of the confluence of the Avon and Heathcote River channels.

The abrupt change in salinity pattern over the tidal cycle which was shown along the eastern slopes presumably indicated a boundary between incoming seawater, and a mixture of fresh and estuary water banked up by the flood tide. This estuary water lowered the salinity on the upper intertidal slopes during periods of windy conditions.

Voller (1973), who sampled salinity along a transect from H.W.L. to L.W.L. near to stations 3 and 4, showed that the greatest range for water at the bottom of the water column was at L.W.L. from 3.2 to 32.0‰ and at H.W.L. the maximum was similar at 29.9‰ but the minimum only dropped to 8.2‰. This study also showed that interstitial salinity was little affected by tidal changes above the sediment, only changing from 26.6 to 26.1‰ during the tidal cycle at H.W.L. Other studies from this estuary have shown some stratification (Kilner 1969) and no stratification (Estcourt 1962) depending on the area examined, and the wind conditions at the time of sampling. The salinity profile at a particular site probably varies considerably according to wind, river flow and peripheral drainage, heavy rain, and high evaporation during emersion on dry windy days. There was no evidence from the results of the present study to suggest that there are consistent and predictable changes in salinity in advance of the flood tide level, which would trigger the burrowing response in A. *crenata*.

2. Sediment particle size

These results show that the silt-clay percentage in the vicinity of stations 1 and 2 decreased between the time of measurements summarised on a sediment map published in 1973 (Knox and

Kilner), and 1974. The 1973 map showed a patchy distribution with a greater spread of silt-clay dominated sediments at about M.W.L. on the western slopes. During the course of the present study the sediments at M.W.L. down to L.W.L. comprised areas of sticky mud on the surface, although generally the sediments on the western flats were firmer than formerly.

The results of the present study agree closely with a recent more detailed study of Avon-Heathcote Estuary sediment (Macpherson 1977). Stations 1 to 4 lie close to his graphic mean diameter contour for 2.5ϕ (2.0 above, and 3.0 below or down the shore) and to the 5 to 20% muddiness contour. In general the north east slopes are about an order of magnitude less muddy than western slopes for the same depth and distance from the river mouth, which he attributed to Avon River input, and not to sewage effluent. Both east and west slopes are undergoing net erosion (Fig.2.1.), predominantly caused by wave energy, although patches of sediment accumulation are described on the lower western slopes and flats. The greater slope above M.W.L. at stations 1 and 2 probably prevents deposition of wave-borne silts from the Avon River settling on the upper slopes. Though stations 3 and 4 have a much lower slope angle, the easterly wind which predominates (Macpherson 1977) is off these slopes and not onto them.

Knox and Kilner (1973), summarising the general work on faunal distribution in Avon-Heathcote Estuary, stated that *A. crenata* distribution is correlated with high silt-clay percentages and high organic content. Size distribution was related to finer sediments in Whangateau Harbour (Briggs 1972) and Watters (1964) stated that *A. crenata* can cope with medium sands but showed a preference for finer grades. Silt content was the only sediment feature which

was found to distinguish the eastern (less than 2% silt) and the western slopes (up to 20% silt). If sediment particle size has a significant effect on the size structure of the *Amphibola* sub-populations in the two areas then this difference in silt content is presumably the determining factor. Any consideration of the effect of the sewage outfalls near to stations 1 and 2 should therefore also take account of silt content in this area. This silt content at stations 1 and 2, however, was considerably lower than that below M.W.L. on the western mud flats, and along the lower reaches of the Avon and Heathcote Rivers where silt content reached over 80% of the sediment. Silt content may affect *Amphibola* size according to a threshold value rather than by a linear relationship, or become significant beyond a particular silt content or size of animal.

3. Organic Content

There was no apparent seasonal pattern resembling that found for organic carbon in some estuaries (Driscoll 1975). Organic content in the Avon-Heathcote Estuary has also been measured by Bruce (1953) who heated mud samples in a crucible over a bunsen burner. Values of 4.73 and 5.88% were recorded for samples collected in the vicinity of stations 1 and 2 on the western slopes. Sediment carbon distribution in the Avon-Heathcote Estuary was plotted by Knox and Kilner (1973) to show 1.0 to 1.75% for the eastern slopes (stations 3 and 4) and L.W.L. on the western flats, and 3.0 to 7.0% on the eastern slopes above M.W.L. Organic carbon content on the eastern slopes seems to have remained constant since this previous work, but levels on the western slopes have obviously decreased, probably in association with recorded changes

in sediment grain size over this area.

The levels of organic matter recorded in this study, for stations 1 and 2, (2.0 to 2.5%), are of a similar order to those (1.45, 1.65, 2.91%) measured by Briggs (1972) in the fine sediments favoured by small *A. crenata* in Whangateau Harbour. It is difficult to separate the significance of organic content from that of the silt content. Densities of benthic microflora (e.g. *Euglena* spp.) increase with decreasing sediment particle size, increasing organic carbon and nitrogen content, and increasing salinity (Steffenson 1974, Munawar 1972). Particle size and organic content are thus important determinants of the food concentration of *Amphibola*, which may support the dense population of small individuals at stations 1 and 2, but does not explain the relatively sparse population of adults in this area.

4. Temperature

Other studies in Avon-Heathcote Estuary have shown that the air temperature range is intermediate between that measured in Christchurch City, and coastal air temperatures (Thompson 1929, Webb 1965, Estcourt 1962). Differences in air temperature of 1 to 3°C across the estuary were noted by Webb and Estcourt, although no specific relationships were described. Estcourt measured minimum estuary water temperatures of 7°C in Jun. and Jul., and maximum temperatures, reaching 27°C, in Dec.,Jan. and Feb. He observed the same range of temperatures in air and sediment. In the present study the seasonal cycle of surface sediment temperature generally showed greater extremes than air or estuarine water temperature. The difference in mean emersion sediment temperatures between the western and eastern slopes is probably related to predominant wind direction. In summer the wind was

generally a cool easterly from the sea which lowered temperatures on the western slopes, but had relatively little effect on the eastern slopes, which were sheltered from this direction. In winter the eastern slopes were exposed to the predominant cold southerly winds.

The slightly higher summer temperatures on the eastern slopes were unlikely to have major effects on size-frequency distribution of *Amphibola* but could cause differences in diurnal growth rates or ripening of the ovotestis.

CHAPTER III

SHELL MORPHOLOGY

I. INTRODUCTION

The shell can provide a readily measurable record of the history of accretionary growth and form of an individual mollusc. The gastropod shell, in particular, has been studied as the expression of a complex mathematical form (Raup 1966, Vermeij 1971 a), environmental influences (Wigham 1975, Crothers 1975), ecological requirements (Linsley 1978, Struhsäker 1968), genetic polymorphism (Giesel 1970, Harman 1970, Luckens 1970), and evolutionary relationships (Cain 1977, Vermeij 1971 b). Shell growth has been considered in terms of overall size (Frank 1965, Seed 1973, Stromgren 1976), allometric relationships between different dimensions (Crothers 1975, Eckbald 1971, Cain 1977), the morphological expression of a logarithmic growth form (Huxley 1962, Vermeij 1971 a, b, 1973), and the physiological and biochemical processes of shell deposition (Lutz 1976, Watabe et 1958, Wada 1961, Wilbur 1960). Shell growth rate and form may show wide intraspecific variation, often giving rise to taxonomic confusion. Harman (1970) described a clinal variation in shell height to width ratios within a lake population of Lymnaea emarginata which may be explained by limited gene flow, environ-

mental stress, or both. Intraspecific variation in shell sculpture of Littorina picta has a genetic basis, but was found to be expressed according to environmental selection processes such as wave action, prolonged submersion, desiccation, high temperature and extreme salinity (Struhsaker 1968). Shell shape in the common dog whelk, Nucella lapillus, described by the ratio of maximum length to aperture length, was found to describe predictably the exposure of the habitat to wave action (Crothers 1975).

Measurements of gastropod shell morphology have been based on general gross dimensions such as shell length and width, geometrical parameters describing the components of shell coiling (Raup 1966, 1967; Vermeij 1971 a), and microcopic examination of the nature of the growth increments which are the structural units of the shell. The geometrical analysis was developed from a logarithmic model of the gastropod shell within a set of x, y, z coordinates. The parameters derived from this model describe each of the vectors of differential growth which determine a particular shell's form. The model recognises the gastropod shell as:

(i) a cone (if uncoiled), which has a height and a base equivalent to the aperture, described by the generating curve; which is coiled in

(ii) an x, y plane, that is a two dimensional spiral, described by the expansion rate; and

(iii) along a z axis, producing a corkscrew or turbinate form, described by the translation rate.

Furthermore, localised deviations in growth rate from the basic logarithmic form produce surface features such as ribs or spines, characteristic of many gastropod shells. These deviations

are the result of increases or decreases in mantle activity at specific sites or times. The mantle is a collar around the gastropod body, posterior to the foot, responsible for shell deposition. Deposition rate is thus a function of mantle area (and therefore soft body size), and rate of secretion, which can be affected by biological and environmental rhythms (Pannella and MacClintock 1968), seasonal and daily changes in factors, such as temperature, or disturbance, and the availability of chemical constituents of shell material (Wilbur 1964). Discrete periods of deposition of calcium carbonate from the mantle result in layers in the shell which have been found to provide a record of tidal, daily, lunar, seasonal, and annual events in bivalves (Pannella and MacClintock 1968). Studies of microscopic growth increments have concentrated exclusively on bivalves, and similar comparative work on sessile barnacles (Bourget 1977), and as far as it is known no similar work on growth lines in gastropods has been described.

In this aspect of the present study the morphology of the shell of *Amphibola* was described by measuring:

(i) general shell linear dimensions;

(ii) geometrical parameters of shell coiling;

(iii) weight-linear relationships, and weight-growth relationships;

and examining

(iv) the microscopic pattern of shell deposition and sculpture.

Appropriate dimensions were related to each other by regression models which allowed the shell characteristics of *Amphibola* occurring at different stations, to be compared. It was postulated that environmental factors affecting size distribution at these stations (Chapter 4) might also be expressed as mopho-

logical differences. Environmental conditions related to the sewage outfalls adjacent to stations 1 and 2 could affect shell morphology through the mantle enzyme systems responsible for shell deposition and calcification, or through genetic perturbation resulting in deviations from the normal coiling form. Chlorinated hydrocarbons have been found to inhibit enzyme systems responsible for deposition of medullary calcium for egg shell by birds (Bitman et al., 1970). Valentine et al. (1973) suggested that sub-lethal levels of industrial pollutants may be the cause of increasing asymmetry in the skeletons of fish in California waters. The accretion of vertebrate skeletal material involves processes of calcium metabolism and transport similar to those of shell deposition in molluscs.

II METHODS

Amphibola were collected from stations 1 to 4 (Fig. 1.1.) during Jun., Jul., Aug., Dec. 1974, and Jun. 1975. A representative size range of shell lengths larger than 5mm was collected, although the dominant size class was 20 to 25mm. Independently collected samples were pooled to give a total for each station of about 50 individuals on each sampling month. These samples were collected as part of a preliminary survey to determine the most effective sample size, sampling technique, and variates for measurement in the subsequent regular sampling programme (Chapter 4).

(1) General Shell Dimensions

Shell dimensions A, B, C, D, F, G (Fig.3.1.) were measured to 0.1mm with sliding calipers. The operculum was measured after removal from the soft body with forceps sharpened to provide oppo-

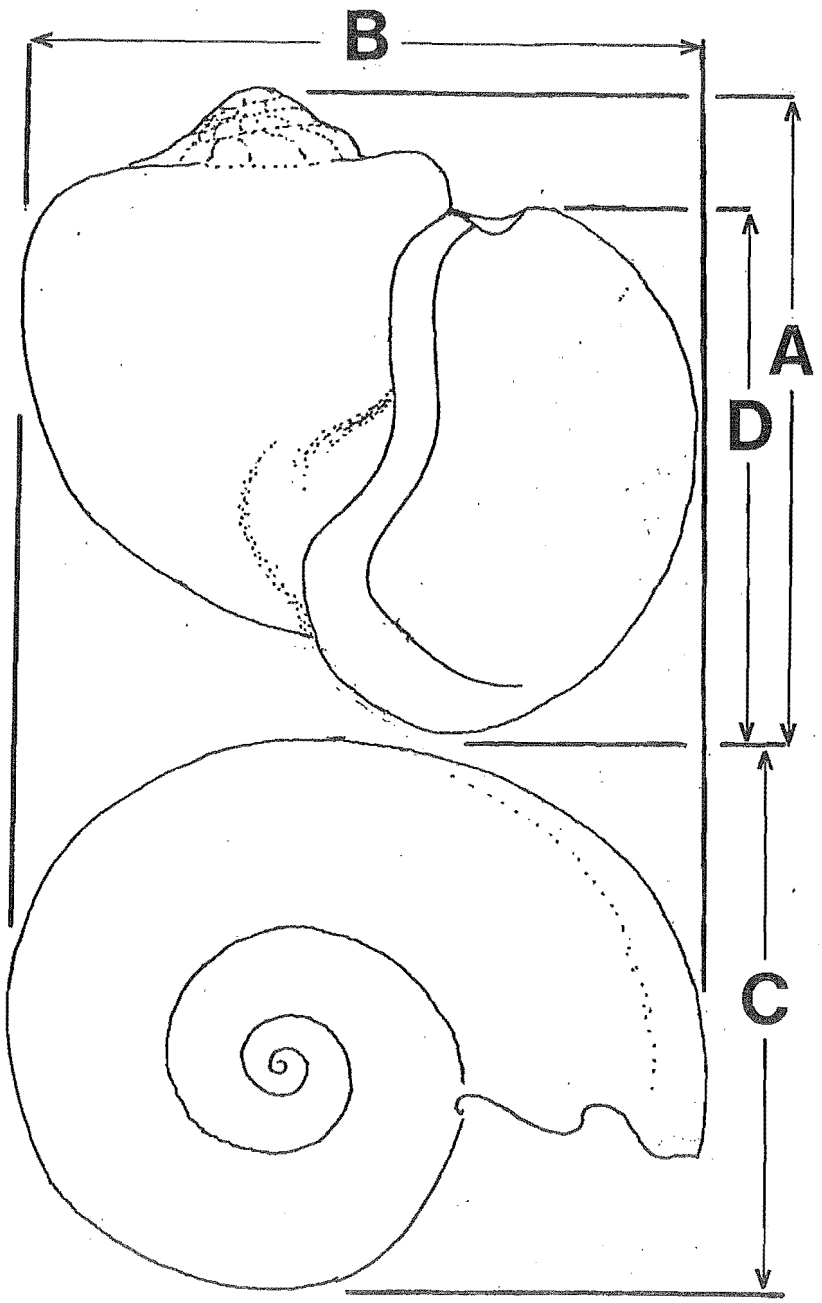


Fig.3.1. Outline of adult *Amphibola crenata* shell showing general shell dimensions: A = shell height
B = maximum shell width
C = minimum shell width
D = aperture length
Also F = operculum length G = operculum width
U = operculum area (π. F/2. G/2)
Spire index, R = A/B; S = A/D; Globosity, T = $C/(AxB)^{\frac{1}{2}}$

sing blades which were used to sever the opercular attachment muscle.

(2) Geometrical Parameters

Shells were mounted with plasticine on a sheet of glass, adapertural view uppermost with the maximum shell width plane parallel to the glass surface. The sheet of glass bearing the specimens was placed flat on a sheet of photographic printing paper and exposed under a photographic enlarger. The light source was positioned at the maximum distance above the shells to ensure a light projection over each shell that was as perpendicular to the glass surface as possible. After processing the printing paper to produce a photogram (Fig. 3.2.) acetate was laid over it and lines drawn to describe dimensions and angles for each individual, for measurement. Expansion rate of the coils ($V = (H/I)^2$), translation rate along the z-axis ($J = \cot('A'/2)$ where 'A' is the apical angle of the shell), and the angle of elevation (K) of the z-axis of coiling above the plane of the aperture, or generating curve, were measured. These parameters follow those developed by Raup (1966, 1967) and Vermeij (1932 a), and are shown in Fig. 3.2. The angle (L) at the spire of the shell (Fig. 3.2.) was also measured.

(3) Weight

Animals were kept for 24h at 15°C after collection to eliminate sediment from their alimentary canals. Algal growth was removed from the shells. Dry weight was measured to 0.01g on an electric balance after animals had been dried at 65°C for 72h in an oven ventilated by a fan. Dry weight included shell, soft body and operculum weight. Dry weights of animals collected dur-

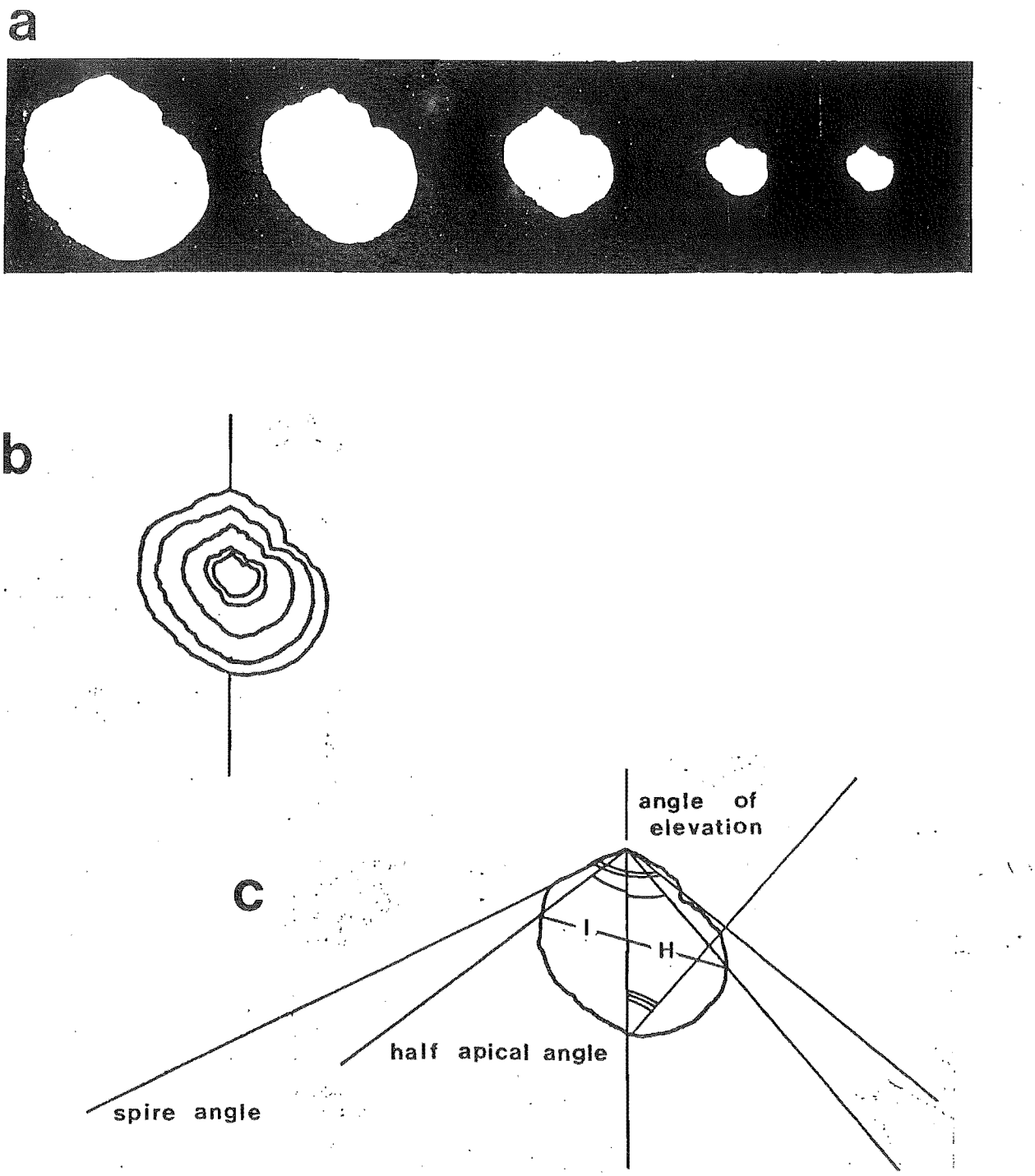


Fig.3.2. Geometrical parameters of *Amphibola crenata*
a. Photograms showing shell shape reduced to two dimensions for measurement of geometrical parameters.
b. Photograms superimposed showing that shell shape is constant over a wide size range i.e. there is no allometric change within this size range.
c. Parameters used to describe coiling geometry
Expansion rate of the coils, $V=(H/I)^2$
Translation rate, J=cot half apical angle
Angle of elevation, K
Angle at the spire of the shell (compare with apical angle) was also measured.

ing a survey of the whole estuary in 1975 (Chapter 4) were pooled to describe the age-weight-growth structure of the whole estuarine population.

Wet soft body weights were measured after shell removal during the preparation of animals for gonad analysis (Chapter 5). The soft body was blotted dry on paper tissue and weighed to 0.01g on an electric balance.

(4) Shell Deposition and Sculpture

The outside aperture lip was removed with bone forceps to provide a section of the most recent shell growth area. This section was cleaned and set in epoxy resin in a vessel, evacuated to minimise bubble entrapment in the resin.

After it was set the resin block was cut with a diamond circular saw to produce a cross section perpendicular to the aperture edge at the point of maximum shell width (Fig. 3.7.). This section was progressively polished on a rotating felt plate using fine polishing powder. Excessive polishing was avoided as it tended to hollow out the softer shell material leaving the hard resin. The shell section was then etched by immersion for 20 sec. in 1% hydrochloric acid, and washed with distilled water. The etched surface was flooded with acetone and a piece of acetate sheet was laid gently over this. After the acetone had dried the hardened acetate was removed and taped to a microscope slide for examination under a compound microscope.

The periodicity of the growth lines was determined by placing freshly collected, live *Amphibola* in a tank containing 10% seawater to a depth of about 5mm. The 10% seawater was made up from open ocean water and distilled water, and to this was added 10ppm alizarin sodium monosulfonate, a vital stain for mollusc

shells, described by Hidu and Hanks (1968). This system was kept in a constant environment cabinet at 15°C, and the seawater and stain medium was changed daily. After staining for at least one week (Table 3.III) eight animals were places in each of two cages at each station (1 to 4) on the estuarine mud flat. Each cage comprised a frame 500mm x 500mm square and 150mm high covered on 4 sides and top with plastic mesh and held by stakes driven into the sediment. The lower edge of the frame walls were buried 50mm below the sediment surface. After a given growth period (Table 3.3) the *Amphibola* were removed from the cages and prepared for examination of the growth lines according to the procedure above. Unfortunately the intended comparison between stations was not possible because of losses and interference to cages.

III RESULTS

(1) General Shell Dimensions

Shell length and maximum shell width were very similar to one another as were minimum shell width and maximum aperture (Table 3.I). In each case the mean value was about 5% less for stations 3 and 4 than that for stations 1 and 2. These major dimensions were used to calculate ratios which summarise the overall shape of gastropods: spire index, R= length to width ratio (Cain 1977); shell length to aperture length ratio, S (Crothers, 1975); and shell globosity, T (Vermeij 1973) using minimum width as an approximation to shell height when the animal is active on the sand surface (Fig. 3.1.). Mean values for these indices were R (0.977 to 0.986), S (1.219 to 1.237) and T (0.769 to 0.791) Table 3.I). Analysis of variance showed that there was a significant difference (p. less than 0.05) between

Table 3.1 Comparison of means of variates describing shell shape and weight at stations 1 to 4.
S1, S2, S3, S4 are stations 1 to 4 (i.e. S1 to S4) respectively
(S12) is the combined data from stations 1 and 3
(S34) is the combined data from stations 3 and 4

See facing page for key to variates.

Variate		A	B	C	D	R	S	T
n		1148	1148	1148	1148	1148	1148	1148
means	S1	23.846	24.602	19.219	19.558	0.973	1.218	0.793
	S2	23.087	23.594	18.146	18.854	0.981	1.220	0.789
	S3	22.127	22.478	17.137	17.977	0.985	1.231	0.769
	S4	22.301	22.616	17.208	17.927	0.987	1.243	0.768
	(S12)	23.466	24.098	18.682	19.206	0.977	1.219	0.791
	(S.D.)	(4.814)	(4.966)	(4.022)	(3.747)	(0.054)	(0.006)	(0.023)
	(S34)	22.214	22.547	17.172	17.952	0.986	1.237	0.769
	(S.D.)	(2.541)	(2.528)	(1.881)	(1.883)	(0.048)	(0.056)	(0.028)
p. S1 to S4		0.000	0.000	0.000	0.000	0.015	0.000	0.000
p. (S12)(S34)		0.228	0.075	0.010	0.040	0.165	0.086	0.175

Variate		E	F	U	G	J	K	L	V
n		377	377	377	377	585	585	585	585
means	S1	4.041	14.416	97.342	8.540	0.892	35.958	120.45	1.612
	S2	3.510	13.550	89.832	8.285	0.929	37.258	118.56	1.339
	S3	2.139	12.598	75.134	7.482	0.921	37.458	120.34	1.511
	S4	2.101	12.565	75.254	7.480	0.927	35.609	120.01	1.366
	(S12)	3.775	13.983	93.587	8.412	0.911	36.608	119.51	1.475
	(S.D.)					(0.102)	(4.085)	(9.380)	(0.563)
	(S34)	2.120	12.581	75.194	7.481	0.924	36.534	120.18	1.439
	(S.D.)					(0.085)	(3.218)	(6.887)	(0.332)
p. S1 to S4						0.001	0.634	0.045	0.000
p. (S12)(S34)						0.121	0.820	0.351	0.343

n is the number of observations
S.D., i.e. standard deviation, $S = (\Sigma(x - \bar{x})^2/n)^{\frac{1}{2}}$

p. S1 to S4 is the probability of the difference between stations being be chance.
p. (S12)(S34) is the probability of the difference between areas being by chance.

stations for the shell dimensions, A , B, C, D, R, S, T, but that when adjacent stations were combined, (S12) and (s34), the increased deviations for each mean resulted in no significant difference for variates A, B and D and their derivatives.

Correlation coefficients and regression lines of pairs of relevant variates were computed and compared by covariance analysis (Table 3.2). A simple linear least-squares regression model was found to fit the data points well (Appendix I) and account for more than 75% of the variation in all cases (Table 3.11). All variates were subject to measurement error andtherefore did not provide pairs of independent and dependent variates. For this reason, regressions of x on y, and of y on x, were computed for reference and are shown in Appendix I.

A high correlation (greater than 0.9) was found between linear shell dimensions, and after double log transformation, between shell length (and width), and operculum and weight measurement (Table 3.11). Correlation for each pair of untransformed variates was significantly different between the two areas with stations 1 and 2 having a higher coefficient. The situation was reversed for correlation between shell length and opercular length and area. At stations 1 and 2 with older individuals the opercula were larger and more variable because they were often damaged or eroded. Assuming the ovate operculum to approximate an ellipse, the area of the operculum was calculated using the length and width of the operculum as major and minor diameters respectively, i.e. area of operculum = π x operculum length/2 x operculum width/2 opercular area was related to shell length (b = 1.742), shell width (b = 1.821), and aperture length (b = 1.910) using a double log transformation. There was no correlation between shell length

Table 3.11 Comparison of correlation coefficients and regression lines of variates describing shell shape and weight at stations 1 to 4.
(S12) is the combined data from stations 1 and 3
(S34) is the combined data from stations 3 and 4
Overall S1 to S4 is the combined data from stations 1 to 4

	AB	AC	BC	AD	BD	AE
Appendix I graphs	1	2	3	4	5	6
Correlation (R)						Log, log
R, (S12)	0.962	0.979	0.981	0.964	0.983	0.954
R, (S34)	0.931	0.933	0.930	0.921	0.939	0.929
p (same)	0.000	0.000	0.000	0.000	0.000	0.041
R, overall (S1 to S4)	0.961	0.966	0.970	0.958	0.974	0.948
%ss	92.42	93.36	94.19	91.80	94.94	89.92
Regression (y = a + bx)	B = a + bA	C = a + bA	C = a + bB	D = a + bD	D = a + bB	E = a + bA
a	0.606	0.121	0.261	1.379	1.307	-10.275
b	0.992	0.778	0.757	0.751	0.741	3.547

	BE	DF	FG	AU	BU	DU
Appendix I graphs	7	8	9	10	11	12
Correlation (R)	Log, log	Log, log	Log, log	Log, log	Log, log	Log, log
R, (S12)	0.933	0.834	0.759	0.737	0.748	0.721
R, (S34)	0.928	0.902	0.883	0.903	0.928	0.894
p (same)	0.742	0.009	0.000	0.000	0.000	0.000
R, overall (S1 to S4)	0.948	0.915	0.874	0.890	0.904	0.885
%ss	89.95	83.78	76.44	79.27	81.81	78.34
Regression (y = a + bx)	E = a + bB	F = a + bD	G = a + bF	U = a + bA	U = a + bB	U = a + bD
a	-10.688	-0.254	-0.482	-1.104	-1.396	-1.243
b	3.651	0.959	+0.986	1.742	1.821	1.910

	AR	AS	AT	AJ	AK	AL	AY
Appendix I graphs	13	14	15	16a	16b	16c	16d
Correlation (R)							
R, (S12)	0.047(n.s.)	0.402	0.117	0.313	0.536	-0.077(n.s.)	
R, overall (S1 to S4)	0.075	0.336	0.007	0.286	0.362	-0.097	-0.424
%ss	0.56	11.27	0.005	8.152	13.096	0.939	17.990

p (same) is the probability of the difference between correlation coefficients of S12 and S34 being by chance..

%ss is the % of the variation (sums of squares) accounted for by the correlation and regression line.

and spire index, shell length to aperture length ratio, or globosity, and it appears that this was because these indices are relatively constant for this species throughout its growth (Appendix I).

(2) Geometrical Parameters

Although reduction of the shell profiles to a two dimensional photogram greatly facilitated measurement of these parameters, it was difficult to ascertain with certainty the position of the z-axis of coiling, and the angle of elevation, K. Mean values for the parameters were translation rate, J (0.892 to 0.929) or apical half angle $48^{o}15'$ to $47^{o}05'$, angle of elevation, K (35.609 to 37.458), and the expansion rate of the whorls, V (1.439 to 1.475). There was some variation between stations for each of these variables but there was no significant difference between areas (S12) and (S34) when adjacent stations were pooled. There was no correlation between shell length and the geometrical parameters (Table 3.II), which was expected as these parameters should be relatively constant for a species (Vermeij 1973 b). Values of each of these variates were clustered around their means and showed no allometric change (Appendix I).

The angle to the top of the shell spire, L, had a mean range of 118.56^{o} to $120/46^{o}$. It was subject to measurement error because the apex of the shell was often irregular and eroded. There was no correlation between this angle and shell size.

(3) Weight

(i) Dry weight - shell length regression.

Dry weight, E, like the opercular dimensions, required double log transformations to produce a linear relationship with shell length, A.

$\log_e E = -10.275 + 3.547 \log_e A$

$\log_e A = 2.920 + 0.253 \log_e E$

Mean dry weight was higher at stations 1 and 2 (3.77g) than at stations 3 and 4 (2.12g). This large weight difference corresponded to a small difference in shell length between the two areas because weight increased exponentially with linear dimensions.

(ii) Dry weight frequency distribution.

Dry weight of *Amphibola* continued to increase as the animal grew older (Appendix I, 9) whereas rate of change in shell length decreased with age. In a species with a discrete annual breeding season and uniform growth rates individuals year classes retain their identity as distinct modes in a size-frequency distribution (Franz 1971) *Amphibola* collected at stations 1 to 4 did not provide a representative sample of all size classes (Chapter 4) and so dry weights of animals collected along line transects covering the range of *Amphibola* in the Avon-Heathcote Estuary were pooled (Fig. 3.3.). The largest mode comprised very small newly settled individuals, but modes (or plateaus) also occurred at 1.0, 2.5, 3.5, 4.4, 4.7, 5.3 and 5.7g. Very occasional individuals were found which weighed more than 6.0g. The weights produced sigmoid growth curve when plotted against year class.

(iii) Soft body weight.

Soft body weight plotted aginst shell length produced a linear equation after double log transformation (Fig. 3.4.), with a higher correlation coefficient (0.92) than the raw data (Table 3.3). There was no significant difference between correlation coefficients for combined data from stations 1 and 2, and from stations 3 and 4. The slopes of the regression lines, however, were significantly different between areas ($p = 0.002, 0.003$),

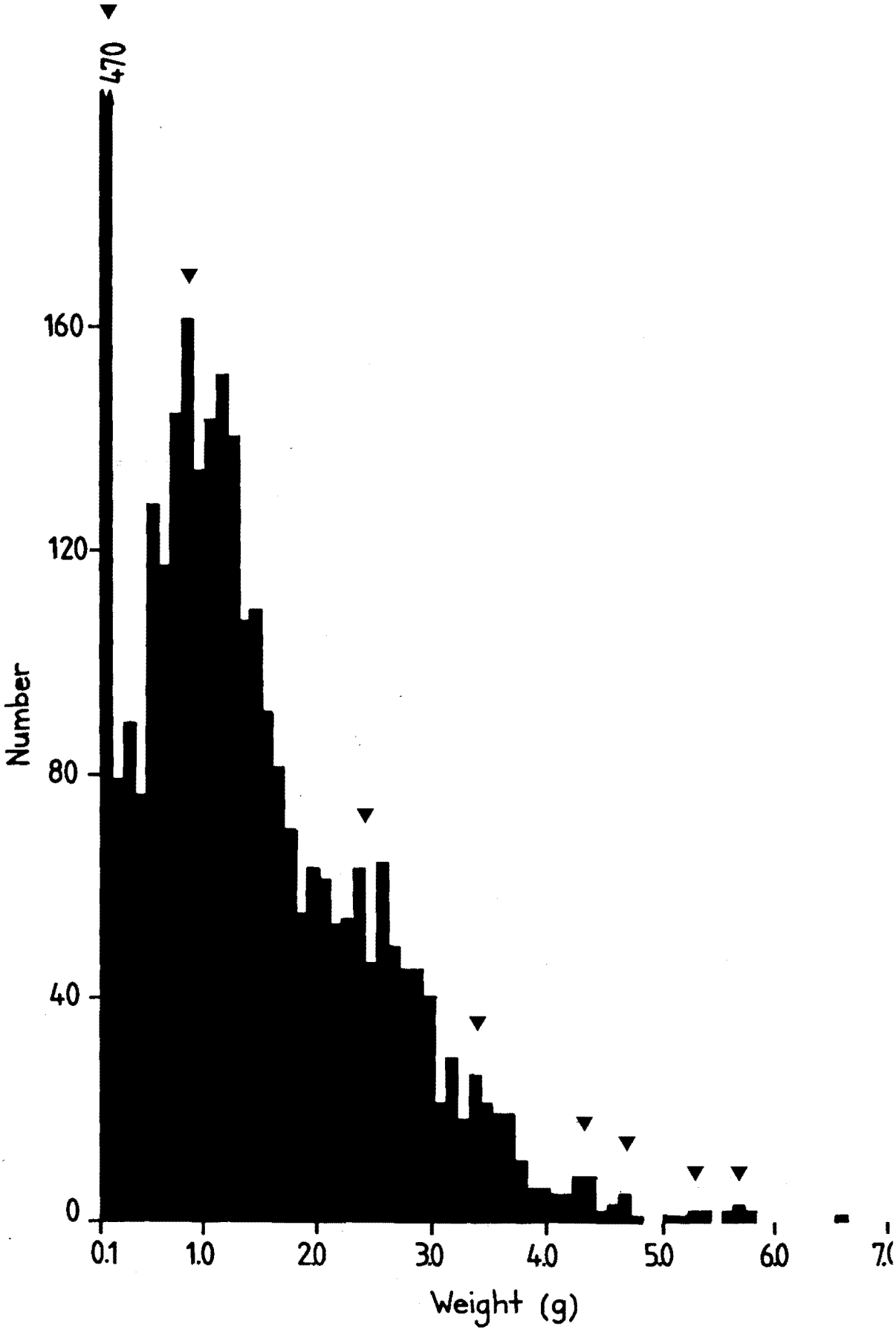


Fig.3.3. Dry weight frequency of *Amphibola crenata* collected from throughout the range of this species in the Avon-Heathcote Estuary. Samples were collected during winter in 1975. Modes, or plateaus are indicated by an arrow, and may represent year classes.

Fig.3.4.(facing) Soft body weight (M) plotted against shell length (A); a, for raw data, and b.,c. after double log transformation. The effect of larger body size at stations 1 and 2 is shown by the different slopes of the regression lines for combined data from each pair of stations.

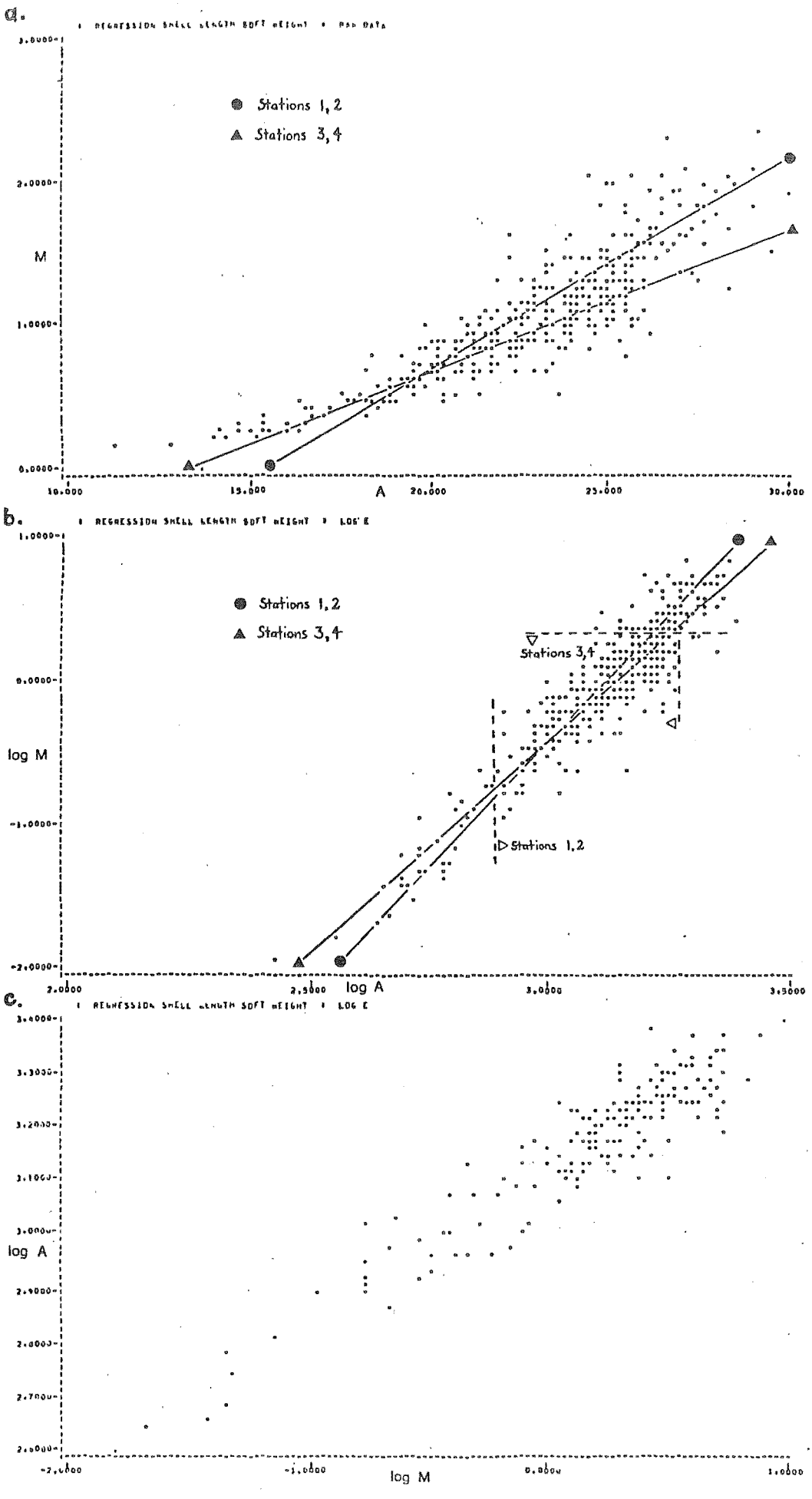

a.
Stations 1, 2
Stations 3, 4
M
A
b.
Stations 1, 2
Stations 3, 4
Stations 3,4
Stations 1,2
log M
log A
c.
log A
log M

Table 3.III Comparison of correlation coefficients and regression lines for shell length (A), soft weight (M), at stations 1, 2 and 3, 4

	stations 1,2	stations 3,4	Total
Correlation (R)			
Raw data	0.876	0.878	
Log, Log trans.	0.927	0.924	0.931
%S.S. log, log	86.003	85.412	

Difference between R(log,log), stations 1,2 and 3,4; $p \doteq 0.830$

Regression ($y = a + bx$)

M = a + bA (log,log)

a	-9.875	-8.710	-9.470
b	3.168	2.766	3.028

A = a + bM (log,log)

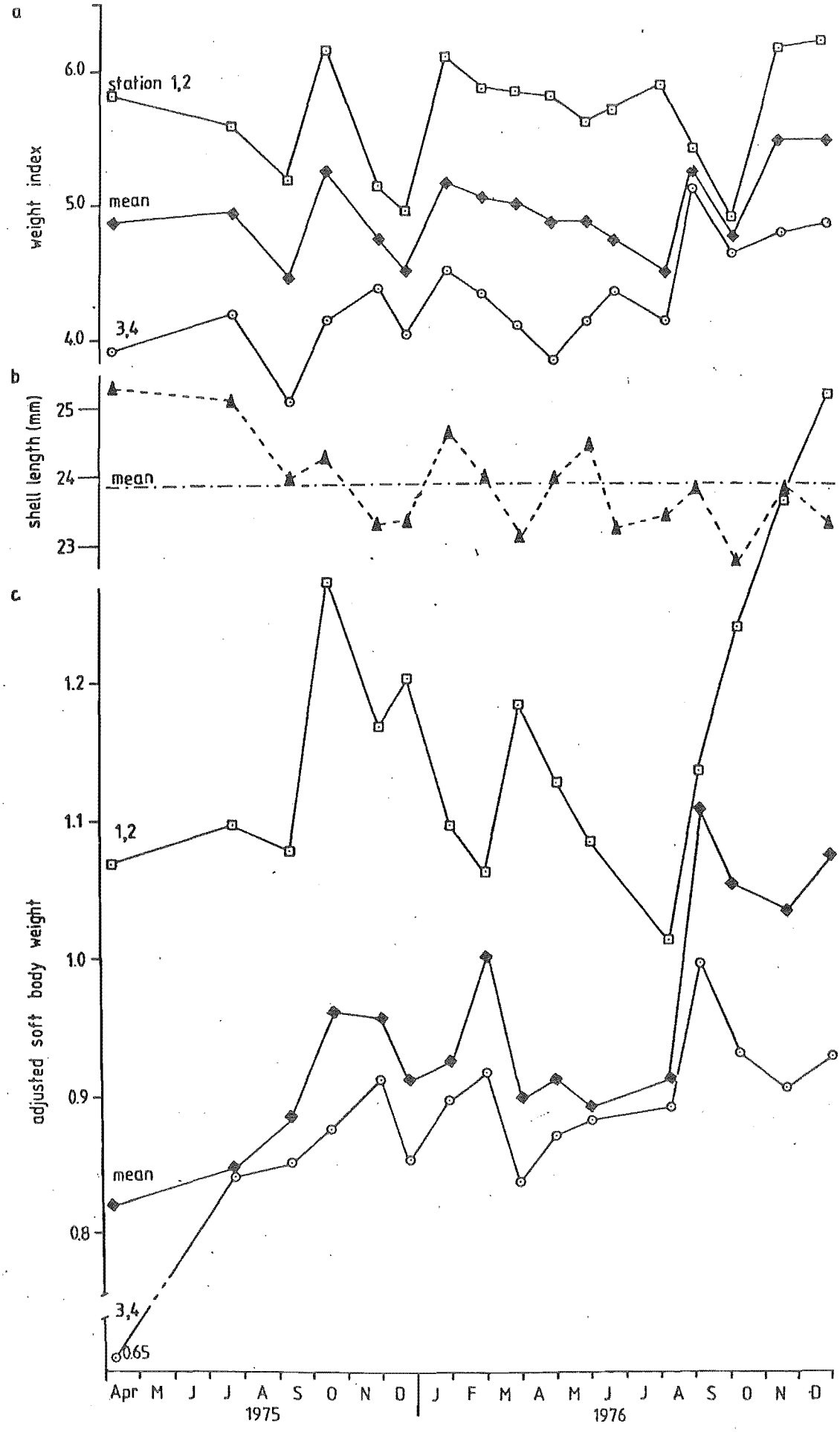
a	3.124	3.137
b	0.271	0.309

Covariance analysis of difference between slopes of lines for stations 1, 2 and 3, 4

M = a + bA $p = 0.003$

A = a + bM $p = 0.002$

Fig.3.5.(facing) a. Weight index (soft weight/shell length x 100), b. shell length, and c. adjusted soft body weight (for shell length), each month during 1975 and 1976.


a
weight index
6.0
5.0
4.0
station 1,2
mean
3,4
b
shell length (mm)
25
24
23
mean
c
adjusted soft body weight
1.2
1.1
1.0
0.9
0.8
1,2
mean
3,4
0.65
Apr M J J A S O N D J F M A M J J A S O N D
1975
1976

resulting from a difference in range of values in each area (Fig. 3.4.). Slopes (b) of regression lines for shell length, and dry weight (shell and soft body), and wet soft body weight (after transformation) were 3.547 and 3.168 respectively.

Soft body weight is often affected by seasonal changes, such as storage of lipid in winter, high food availability in spring, and gonad increase prior to the breeding season with a subsequent decrease after discharge of sexual products (Ansell 1974). Soft body weight was highly correlated with shell length and therefore seasonal changes in weight may be obscured by differences in mean shell length between samples. A weight index (soft weight/ shell length x 100) was plotted for each month (Fig. 3.5a) but weight index was also correlated with shell length. Mean shell length each month (Fig. 3.5b) was used to calculate adjusted soft body weights (Sokal and Rohlf 1969, p. 443) (Fig. 3.5c). This showed a winter minimum for soft weight in 1975 and 1976, with maxima in spring (Oct.1975), and autumn (Mar., Apr. 1976). Adjusted weight at stations 1 and 2 increased to a very high maximum in Dec. 1976 but this was the result of a few very large individuals in this sample and this trend was not expressed to the same extent in the monthly mean of all samples.

(4) Shell Deposition

(i) Growth lines.

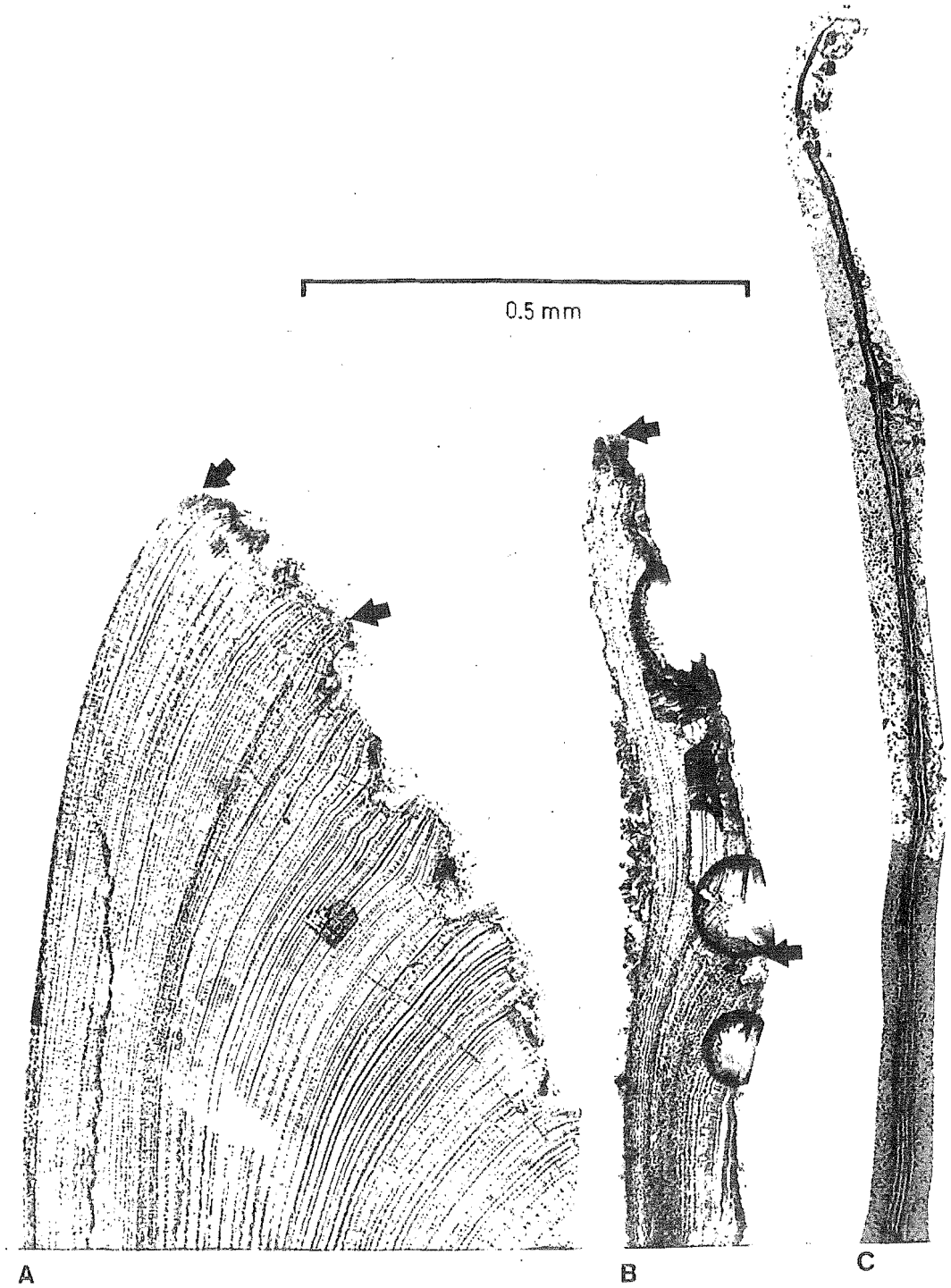
A cross section of shell observed under a binocular microscope showed lines of different opacity of white, sometimes distinguished by pink to violet colouration. These lines were each found to comprise zones which etched differentially to produce a profile in an acetate peel. In individuals of shell length greater than about 22mm (Fig. 3.6A) these lines were

Table 3.IV New shell added during growth experiment. Oct. 1975 to Jan. 1976

Fig. reference (Fig. 3.7)	A	B	C	D	E	F
Shell length (mm)	22.5	12.7	7.4	16.3	17.9	18.3
Animal dry wt (g)	3.61	0.54	0.12	1.22	1.29	1.79
Dates - staining	23 to 3	23 Oct. to 3 Nov.		27 Nov. to 12 Dec.	27 to 12	27 to 12
Dates - growth	3 Nov. to 27 Nov.	3 Nov. to 27 Nov.	Sep	12 Dec. to 9 Jan.	12 to 9	12 to 9
Growth period (days)	24	24	5	24	28	28
Shell added:						
Length (mm)	0.23	0.58	1.3	1.35	2.58	2.0
Mean thickness (mm)	0.16	0.1	0.015	0.10	0.19	0.19
Area (mm^2)	0.04	0.06	0.02	0.14	0.5	0.4
Rate (mm^2/day)	0.0017	0.0025	0.004	0.005	0.018	0.014
Rate/g dry wt individual	0.0005	0.005	0.03	0.0041	0.014	0.008

Photograph on facing page

Fig.3.6A,B,C. Growth lines at the aperture lip for *Amphibola crenata* of shell lengths 22.5, 12.7, 7.4mm respectively. Photographs show acetate peels prepared from polished and etched shell cross-sections. Artifacts in B. are entrapped bubbles in acetate. Arrows indicate period of growth after staining. Internal surface of the shells face photograph left.


0.5 mm
A
B
C

Photograph on facing page

Fig.3.6D,E,F. Growth lines at the sperture lip for *Amphibola crenata* of shell lengths 1.22, 1.29 and 1.79mm respectively. Photographs show acetate peels prepared from polished and etched shell cross-sections. Arrows indicate period of growth after staining. Growth after 7, 14, 21, and 28 days is shown for shell E. Growth after 7 and 21 days coincided with the formation of sculpture ribs. Internal surface of the shell face photograph left.


0.5 mm
21
14
7
D
E
F

similar in appearance to the daily growth lines shown by Pannella and MacClintock (1968) for the bivalve, Mercenaria mercenaria (L.). In smaller *Amphibola* shells, each growth increment had a greater projection in the direction of aperture lip growth. In the smallest individual examined (Fig. 3.6C); each shell growth line (3mm thick) projected an extension of shell 0.2 to 0.3mm beyond the previous line.

Growth lines formed during each experimental period (Table 3. IV) were related to reference lines stained with alizarin. Alizarin staining was carried out with variable success. Some batches had no identifiable stained growth lines, but a sufficient number of stained shells were found to provide satisfactory results. Growth lines were laid down at approximately daily intervals. Convergence, and divergence into several indistinct lines within one 'day's' growth made it difficult to relate lines to particular days, especially in unstained control specimens with no point of reference. Growth in some stained shells during the period in the field in cages, was compared (Table 3.IV). These comparisons were based on material deposited beyond the point of staining, in the direction of aperture growth, and did not consider layers deposited as internal thickening within the existing shell, or the effect of formation of sculptural ornamentation. One specimen 12.7mm long (Fig. 3.6.B) deposited new material at ten times the dry weight specific rate of another specimen 22.5mm long (Fig. 3.6 A), but the absolute rate of deposition (mm/day) was of the same order of magnitude. Three individuals, 16.3, 17.9, and 18.3mm long (Fig. 3.6 D, E, F) showed a wide range of individual shell deposition rates which did not relate to their order of size.

(ii) Sculpture.

Sculptural ornamentation on the exterior of the shell comprises ribs of greater or lesser prominence (Fig. 4.1.) which run discontinuously across the whorl almost parallel with the external aperture lip. These ribs are restricted to the most recent 180° of the last whorl (i.e. the underside of the shell is smooth), and converge with the aperture lip along the half distal to the spire. Microscopic examination showed that these ribs were produced by temporary reflection of the growth increments followed by an abrupt change to non-reflected growth in the direction of the shell spiral (Fig. 3.6E). The formation of these ribs showed a periodicity of approximately 14 days. In growth from 12 Dec. to 9 Jan., 1976 (Table 3.III) two ribs were formed by a change from reflected to non-reflected growth on approximately 19 Dec. and 2 Jan. (Fig. 3.6D,E,F). These two days coincided with spring tides on full moon and new moon respectively. In unsculptured shells there was no feature which could be consistently related to this periodicity.

IV DISCUSSION

(1) Shell Size and Shape

Relationships between general linear descriptors of shell morphology were found to be isometric within the shell size range examined. Changes in allometry sometimes indicate a change in behaviour or habitat (Wilbur and Owen 1964). Migrations and changes in preferred habitat are believed to occur in *Amphibola* (Chapter 4) but no morphological relationship to these changes was found. While a difference in mean shell length of the adult populations at stations 1 and 2, and stations 3 and 4 made it difficult to compare morphology of *Amphibola* from the two areas, there was no

apparent deviation in form which could be related consistently to the collection site.

Spire index, the simplest description of gastropod shape, was close to unity and did not fit within Cain (1977) who showed a bimodal cluster on either side of unity for Western European freshwater basommatophorans. He showed Archaeogastropoda to be mainly equidimensional, but within a taxon any given value can appear outside of the mode (Cain 1977), and marine species comparable to *Amphibola* were not considered. Vermeij (1973a) consuggested that a decrease in growth rate may be accompanied by an increase in spire height (ratio of shell length to aperture length). There was no evidence for a difference in growth rate at any of the stations, based on spire height. The only available comparison of *Amphibola* shell globosity value of 0.7 was with intertidal, hard-substrate Neritidae which have a range for globosity values of 0.4 to 0.7 which is considered to be high (Vermeij 1973a). Resistance to desiccation or heat stress conferred by an increase in fluid reservoir size in gastropods with a high globosity does not affect an intertidal estuarine animal such as *Amphibola* which is able to burrow into the sediments. A relatively large reservoir may be significant to an operculate pulmonate, however, which is often buried in anaerobic sediments for considerable periods.

The double log relationship between shell linear dimensions and total weight and opercular dimensions indicates that with increasing age in older animals, the later dimensions continued to increase while overall shell length and width remained relatively constant. This increase in weight without increase in linear size is often attributed in molluscs to internal thickening of the aperture lip (Franz 1971). Shell growth lines show

that internal thickening of the shell occurred in large individuals (Fig. 3.6.A) which would imply a concomitant decrease in internal aperture size and therefore possibly a restriction in opercular area. The results show that the opposite is the case with opercular area continuing to increase in size. This apparent anomaly is explained by examination of the inside edge of the aperture lip which projects across the axis of coiling at the base of the shell (Fig. 3.7.), thus producing a much greater growth component than that which is expressed in overall shell width or length. These observations emphasise the inadequacy of describing gastropod population size structure simply on the basis of linear dimensions. The component of increase in shell width or length produced by extension of the aperture lip in the direction of the shell spiral is very small unless the rate of translation or expansion is very high. Operculum size is suggested as a useful measure of growth in those gastropods with an operculum, and it is relevant that opercular rings have been used as an indicator of age in the marine gastropod *Babylonia japonica* (Kubo 1953).

The slope (b = 3.547) of the double log equation relating total dry weight to shell length was compared with Calow's (1975) review of allometric coefficients for length and body weight in various mollusc species. The value for *Amphibola* was relatively higher than that for most equiangular turbinate shells, which tend towards 3 (e.g. *Lymnaea stagnalis*: dry tissue weight and shell length, b = 3.035; shell weight and shell length, b = 2.847). Paine (1971), however, found a value of 3.70 for dry weight and shell length in the herbivorous gastropod, *Tegula funebralis*. The values obtained for *Amphibola* in the present study differed from those in Calow's list in that shell and soft body weight were com-

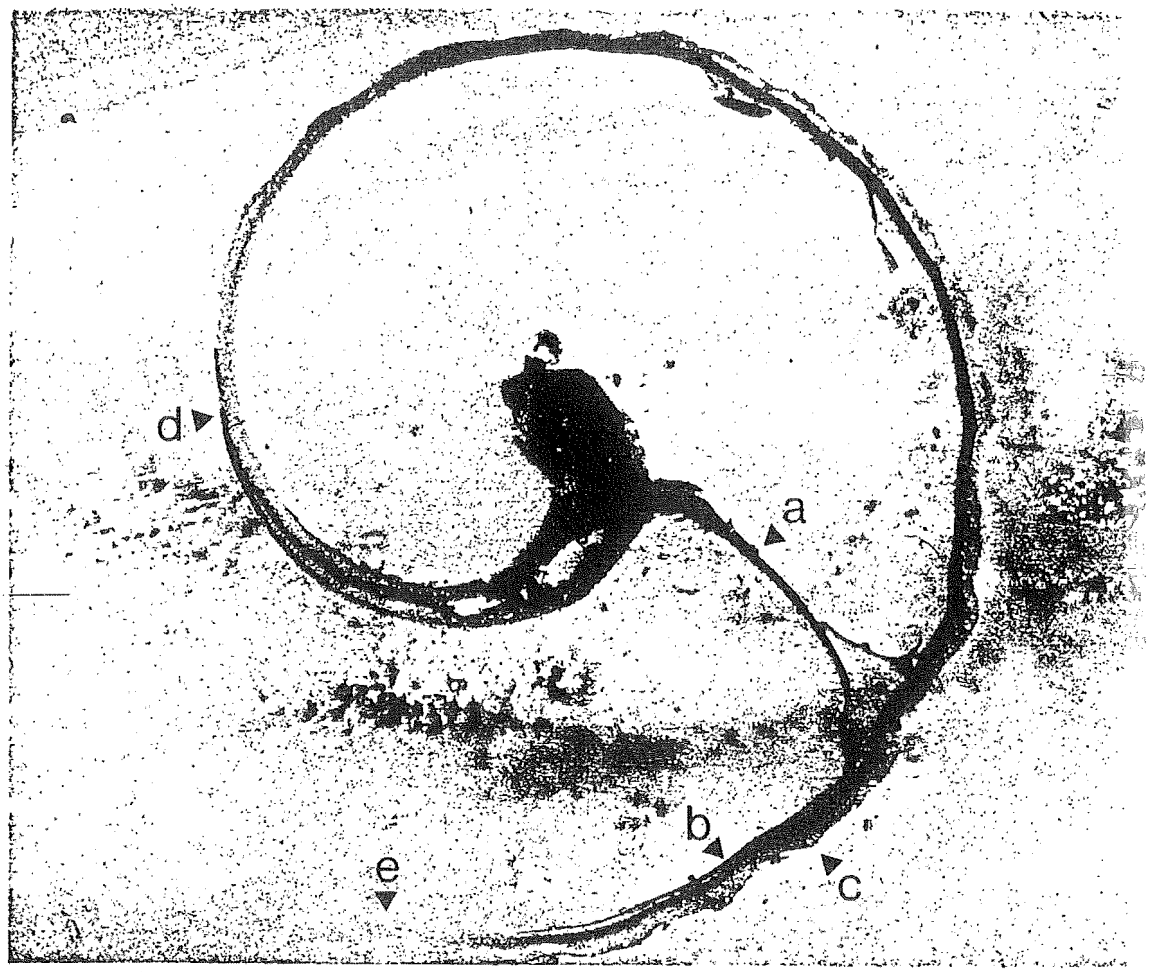


Fig.3.7.. Cross section of the last whorl of *Amphibola crenata* shell of shell width 11.7mm, showing the relatively low rate of expansion of the spiral. The operculum (a) has been preserved in position. The internal shell surface (b), external sculptured surface (c), and the extent of aperture growth on the internal circumference (d) and external perimeter(e) are shown.

bined in a total weight.

Soft body weight did not show a large increase with gonad development, as is shown in many bivalves, e.g. *Chlamys septemradiata* (Ansell 1974). Seasonal weight increases in *Amphibola* gonad are presumably restricted by the location of the ovotestis in the confines of the shell spire. Increase in gonad follicle size was also partly compensated by a reduction in interfollicular cells (Chapter 5). Discrete modes were difficult to isolate from the pooled dry weight frequency distribution of *Amphibola* from the Avon-Heathcote Estuary. The modes therefore did not justify a probability analysis but seven annual modes were indicated which fitted a sigmoid growth curve. On this basis it is suggested that *Amphibola* has a life span of at least seven years in this estuary. Watters (1964), using shell length identified four modes up to maximum shell length of 26.0mm. Briggs (1972), in a more detailed study of growth in *Amphibola*, but also using shell length rather than weight, defined 4 shell length modes up to 27.8mm, and a further 6 modes up to a maximum length of 31.7mm. Briggs' maximum sizes were larger than those found in the Avon-Heathcote Estuary, which may indicate that younger age-size relationships are not directly comparable. Growth line studies indicated that for a brief period (28 days) of summer growth, increase in shell material was quite variable in relation to animal size. This variability indicates one of the underlying reasons for the lack of clear size modes in older age classes.

(2) Geometry, Growth Increments and Sculpture

Vermeij (1973b) has related low translation rates (i.e. high apical half angle, high expansion rates, and ovate to round aperture shape to open shore habitats with a high degree of water movement.

The values for these parameters in *Amphibola*, when compared with those obtained for gastropods in rocky intertidal molluscan communities (Vermeij 1973b) show a very low translation rate (0.9), a high apical half angle (4.7), medium expansion rate (1.5) and an ovate aperture. These features suggest that high gravitational stability is of adaptive importance to *Amphibola* (Vermeij 1973b). A relatively high shell globosity value, is a disadvantage as a profile for a burrowing snail, but also a requirement for gravitational stability. It appears therefore that the estuarine environment of *Amphibola* requires a compromise between a large internal reservoir conferred by high globosity, and the gravitational stability gained through its particular coiling geometry. Although *Amphibola* is not subject to a demand for high stability during tidally-exposed surface activity, the sediment surface is subject to high turbulence from rigorous erosive processes of waves and currents during tidal submersion.

The appearance of daily increments of shell growth deposited by *Amphibola* indicates that the deposition pattern by the gastropod mantle is similar to that shown in studies of bivalve growth. The pattern of deposition during the life of an individual appears to change from non-reflected growth in young snails with a high growth rate, to predominantly reflected growth in older snails. Sculptural ornamentation is the result of a periodic cycle of non-reflected and reflected growth. Rib formation coincided with a 14-day periodicity based on a lunar tidal rhythm. This periodicity has been observed in bivalves, expressed in the widths of growth lines (Pannella and MacClintock 1968). Lunar phases, expressed as tidal events, provide an appropriate basis for maintaining endogenous rhythm in an intertidal organism where the required perio-

dicity of a process is intermediate between diurnal and seasonal. The deposition of shell material in *Amphibola* is probably based on a periodicity influenced by daily tidal, diurnal, lunar, and seasonal cycles as well as the physiological state and age of the individual. A synchronous daily rhythm of digestion in the digestive diverticula and a tidal rhythm of activity and inactivity has been reported for *Amphibola* (Morton 1975) and it is likely that the cycle of mantle activity is related in some way to general body activity. It is not known however, whether shell is deposited during the period of surface locomotion, or during the period spent buried beneath the sediment surface.

Ornamentation of the shell requires a deviation from maximum increase in new shell material in the direction of shell coiling, with a consequent sacrifice in aperture extension or increase in shell volume. An ecological advantage for this expenditure of energy and resources on sculpture may be related to the burrowing habit of *Amphibola*. *Amphibola* in some areas of the estuary burrow into the sediment immediately prior to tidal immersion. Struhsaker (1968) observed that the smooth form of *Littorina picta* was found where water turbulence was greatest but in this situation the shell was on a hard substrate fully exposed to water flow where sculpture is a disadvantage. *Amphibola* has overcome this problem by burrowing into the substrate. Burrowing in gastropods is normally achieved by extending the foot into the sediment and creating a sufficiently fluid area of sand to permit the shell to be drawn beneath the surface (Trueman and Ansell 1969, Trueman and Brown 1976). Sculpture may assist in the burrowing action by agitating the sand and affecting its viscosity around the shell. Once in position in the sediment the ribs projecting into the substrate may reduce the

tendency for water currents to scour around the shell and dislodge it. The shell ribs may also physically strengthen the shell.

It is therefore proposed that a major factor affecting *Amphibola* morphology is the need to resist dislocation on the estuarine mud flat into unfavourable water or sediment conditions. The form of the shell, its coiling geometry, and its sculptural features are maintained by selection for an ability to maintain position on the shore.

CHAPTER IV

POPULATION SIZE STRUCTURE AND DISTRIBUTION

I. INTRODUCTION

Population distribution patterns of a species reflect physiological, ecological and behavioural mechanisms directed towards maximising resource use, reproductive success, and survival. Ecological mechanisms include physical and density dependent pressures, as well as predator or prey distributions.

Intraspecific size distribution is related to tidal level in a variety of unrelated intertidal groups such as chitons (Boyle 1970), polychaetes (Dales 1952), and soft substrate (Edwards 1969) and hard substrate species of gastropods (Vermeij 1972, Bertness 1977). From these studies it appears that a universal trend does not exist from H.W.L. to L.W.L. but a species distribution depends on whether the species is characteristic of the upper or intertidal zone (Vermeij 1972) or on some other factor such as changing food source with increasing size (Bertness 1977).

Past field studies of *Amphibola* refer to apparent size differences between individuals living at different tidal levels. In Hooper's Inlet in Otago, Watters (1964) found that the area of highest organic content (3.3%) sustained the highest density (128 individuals/m^2) of small *Amphibola* and that these small

individuals were associated with large rocks and stones. This habitat preference may be explained by Farnie's (1924) laboratory observation that veligers settle on firm surfaces. Watters found that there was a general size increase from H.W.L. towards the centre of the inlet, and concluded that tidal level was the main factor influencing size distribution. In an inlet in Northland, Briggs (1972) compared size frequencies in a H.W.L. area of fine sediments, high organic content associated with rush beds and carrying a high density of small *Amphibola*, with an area of sandy sediments carrying a lower density of larger individuals. Briggs considered that substrate size was the most important factor in determining size distribution, and suggested that spat settle predominantly on fine substrate. He showed that the size structure difference between the fine and the sandy sediment areas was maintained by active migration of maturing individuals from the fine to the coarser sediments. Briggs also took a transect across the intertidal zone normal to the shore at Pakiri Estuary and found no significant change in mean size with tide level, and the smallest individuals occurred at M.W.L.

In the Avon-Heathcote Estuary, Kilner(1969) found no *Amphibola* living on sediments close to waste outfalls where organic content was highest, but observed a decrease in average size towards the middle of the tidal range, and towards the middle of the estuary. He suggested that juvenile *Amphibola* were limited to areas in the middle range of distribution in the estuary where exposure, feeding time, salinity and substrate size provide the most favourable conditions. This suggestion is consistent with Vermeij's (1972) hypothesis that the purpose of size gradients is to place sexually immature individuals in the area of best

survival within the species' range. Preliminary field observations by the present author suggested that high densities of juvenile animals were associated with sewage outfalls on the western slopes of the Avon-Heathcote Estuary and occurred at the same tide level as a population of predominantly adult individuals on the eastern slopes of the estuary. The aims of this part of the study were

(i) to investigate the stability of the size-frequency distributions of the populations at these two sites over at least 12 months;

(ii) to relate egg laying to settlement and size and density changes during the first year's growth;

(iii) to compare the size differences at the two sites with size-frequency distributions from other areas of the estuary; and

(iv) to investigate the distribution of shells with different degrees of sculpture, around the whole estuary.

II METHODS

(1) Field Sampling

(i) Seasonal changes in size-frequency distribution at stations 1 to 4.

Samples were taken during winter (Jul.) and summer (Dec.) of 1974, and at approximately monthly intervals at each of stations 1 to 4 (Fig. 1.1) from Jun. 1975 to Dec. 1976. Sampling was always carried out when the station was exposed, and during day±1 light hours. Two samples were taken at each station and each was defined by a square wire quadrat of size 0.8m, thrown randomly within an area 20m parallel to the shore by 5m normal to the shore, permanently marked with stakes. The upper 10mm of sub-

strate was removed from the quadrat with a trowel, to fill two 4ℓ plastic buckets. The substrate was then washed through a nest of two Endicott sieves with square mesh pores of 5mm and 1mm diameter, using a fine jet of fresh water from a hose. Samples were sorted fresh, and shell length of retained *Amphibola* was measured to 0.1mm with sliding calipers. A high 'correlation' has been shown between shell length and other linear dimensions (Chapter 3). Shell length was always measured because it was the easiest to measure consistently, and it was taken as the measure of shell size throughout this section. In Apr. 1977 at the end of the sampling period, the number of newly settled individuals recovered by a third sieve of 0.5mm pore diameter was examined.

Before wet weight was measured animals were left 24h at 15°C to eliminate sediment from their alimentary canals. Algal growth was removed from the shells, and the foot was then induced to withdraw to expel excess water from the shell cavity. The shell, foot and operculum were blotted dry with the corner of a tissue, and each animal was weighed to 0.01g on an electric balance. Dry weight was measured on the same balance after animals had been dried at 65°C for 72h in an oven ventilated with a fan. Wet and dry weights included both shell weight and soft weight.

(ii) Egg laying.

The presence of egg clusters or nidi was noted on each sampling occasion and during Jan., Feb. 1975 nidus density was measured at stations 1 to 4.

(iii) Comparison of size-frequencies at other areas of the estuary.

Preliminary observations at stations 1 to 4 showed that

juveniles greater than 0.1mm long were settling by Apr. following their brief summer planktonic life. Thereafter they maintained relatively stable numbers until Jul. This latter period was chosen therefore for comparative sampling surveys. During Apr. to Jun. 1975, samples were taken along line transects from H.W.L. to L.W.L., covering the full estuarine range of *Amphibola* in the lower reaches of the Avon and Heathcote Rivers. Sites of equivalent exposure time along the transects were established by observing the time and level of H.W.L. on that particular day and following the ebbing tide. Samples were taken at H.W.L. and at the water's edge after intervals of approximately one and a half hours, down to L.W.L. No account was taken of spring or neap tide levels as the H.W.L. tended to be modified by embankments, infill, and storm-deposited dead shells and driftwood. During Apr. to Jun. 1976, samples were taken at 5m intervals along a transect from H.W.L. above station 1 to M.W.L., and at 10m intervals along a transect from H.W.L. above station 3, down to station 3. Samples collected during these comparative surveys in 1975 and 1976 were sieved, and the animals measured and weighed using the same methods as described earlier for the regular sampling programme at stations 1 to 4.

(iv) Shell sculpture.

A sculpture index was established by separating the natural range of sculpture from smooth to heaviest sculpturing into 5 arbitrary divisions (Fig. 4.1). Shells collected each month at stations 1 to 4, and at other areas of the estuary during the survey above were compared to this scale and assigned a value from 0 (smooth) to 4.

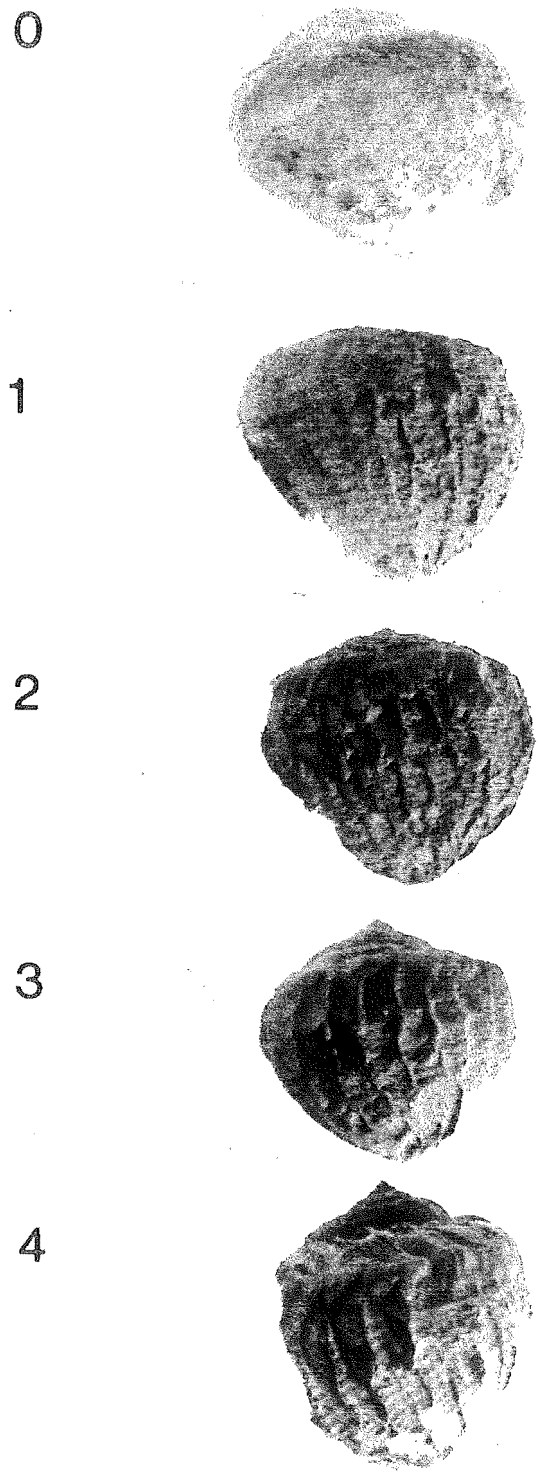


Fig. 4.1. *Amphibola* showing the range of shell sculpture used to establish a sculpture index from 0 (smooth) to 4.

(2) Sediment Preference

Sediment preference was investigated directly by laboratory preference experiments, and indirectly by measuring the grain size of sediment ingested at different stations.

(i) Sediment preference under laboratory conditions.

Surface sediment was collected from stations 1 and 3 and placed separately in either end of a tray, measuring 400mm long by 250mm wide. The sediment was levelled out to a depth of 20mm evenly across the tray and a plastic barrier was placed across the middle of the tray to separate the two sediment types. The barrier just showed at the sediment surface and was intended to minimise mixing of the sediment types during the course of the experiment but allow free movement of *Amphibola*. *Amphibola* were collected from station 1, station 3, and H.W.L. above station 3 to provide a full size range, and divided into size classes according to shell length measured with calipers. Three trays were set up as described in Table 4.V (Experiments (a), (b), (c).) with equal numbers of individuals facing each of the two sediment types, along the barrier. The trays were then weighed and placed in a constant environment cabinet at 15° C, 10h daylight. After 24h the tray was removed, and the number of animals on each sediment type was counted. Animals were replaced along the barrier and the tray replaced for another trial. The tray was oriented at 180° on alternate trials to eliminate effect of the light source or other factors. Ten 24h trials were carried out between 7 Mar. 1977 and 18 Mar. 1977. After each trial the tray was weighed and weight loss made up by the addition of distilled water to each sediment type.

(ii) Sediment ingestion in the natural habitat.

Amphibola were collected from stations 2,3, and H.W.L. above

station 3, measured with calipers, and all external sediment thoroughly removed by washing. They were then placed in separate petri dishes according to source and shell length size class. The bottom of each petri dish was just covered with 4 ‰ seawater made up from distilled and open ocean water. Dishes with their snails were placed in a constant environment cabinet at 15° C and left for 48h. The animals were then removed from the petri dishes and the egested sediment spread out and examined under a binocular microscope. A random selection of 100 grains was measured in each petri dish using an eyepiece micrometer.

(iii) Sediment ingestion under controlled habitat conditions.

The animals from experiment (ii), were pooled and divided into 4 samples each with a representative selection of different sizes. A cage was placed in position at each of stations 1 to 4. Each cage comprised a frame 500mm x 500mm square by 150mm high covered on 4 sides and top with plastic mesh and held by stakes into the sediment. The lower edge of the frame walls was buried 50mm below the sediment surface. One sample was placed in each cage, and left for 48h. Animals were then removed from each cage, measured, and placed in separate petri dishes according to size class and station. After 48h in a constant environment cabinet at 15° C, the egested sediment was measured as in experiment (ii).

(iv) Natural sediment size.

Surface sediment was collected from stations 1 to 4, and from H.W.L. above station 3, and measured as above.

III RESULTS

(1) Field Sampling

(i) Seasonal changes in size-frequency distributions at stations 1 to 4.

The distinctly different shell length frequency distribution between stations 1 and 2, and between stations 3 and 4, as shown by the plot of the June 1975 samples (Fig. 4.2), was maintained throughout the sampling period (Fig. 4.3). The samples from stations 1 and 2 comprised almost all individuals less than 10mm with several scattered large animals (21 - 28mm), while stations 3 and 4 comprised very few juveniles and mainly individuals slightly smaller (15 - 25mm), than the large ones at stations 1 and 2. The total number per m^2 in June 1975 was approximately three times greater at stations 1 and 2. The pattern and changes in shell length frequency for individuals less than 10mm were consistent between each of the replicate samples and between stations 1 and 2, and stations 3 and 4 during the 18 month sampling period (Fig. 4.3). The number of large individuals at stations 1 and 2 were not plotted as their density was too low to give a reliable result from the same size sample adopted for adequate sampling of the small individuals. Increasing the sampling area for these widely dispersed large individuals would have involved the excavation of large quantities of sediment because of the tendency of *Amphibola* to remain buried, particularly during the winter months. Densities of adults were compared at stations 1 and 2 in January 1975. Each mean density was calculated from 12 random quadrats (Table 4.1) and station 1 was more than twice as high as station 2. On 25 Jun. 1976 4 out of 30 (13.3%) of the large adults collected

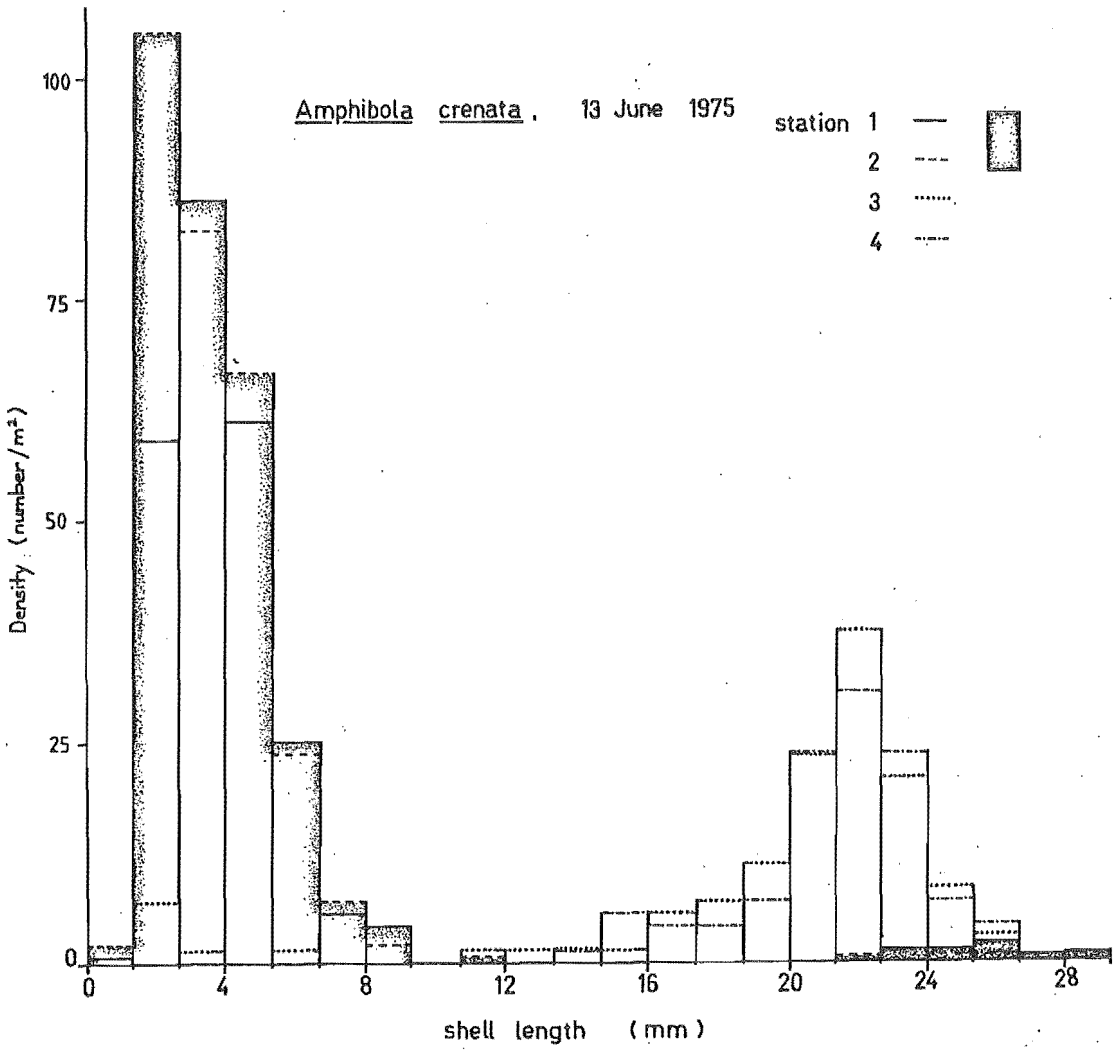


Fig. 4.2 Shell length frequency per m^2 of *Amphibola crenata* at stations 1 to 4 in June 1975. The frequencies for each size class at stations 1 to 4 are superimposed and not cumulative.

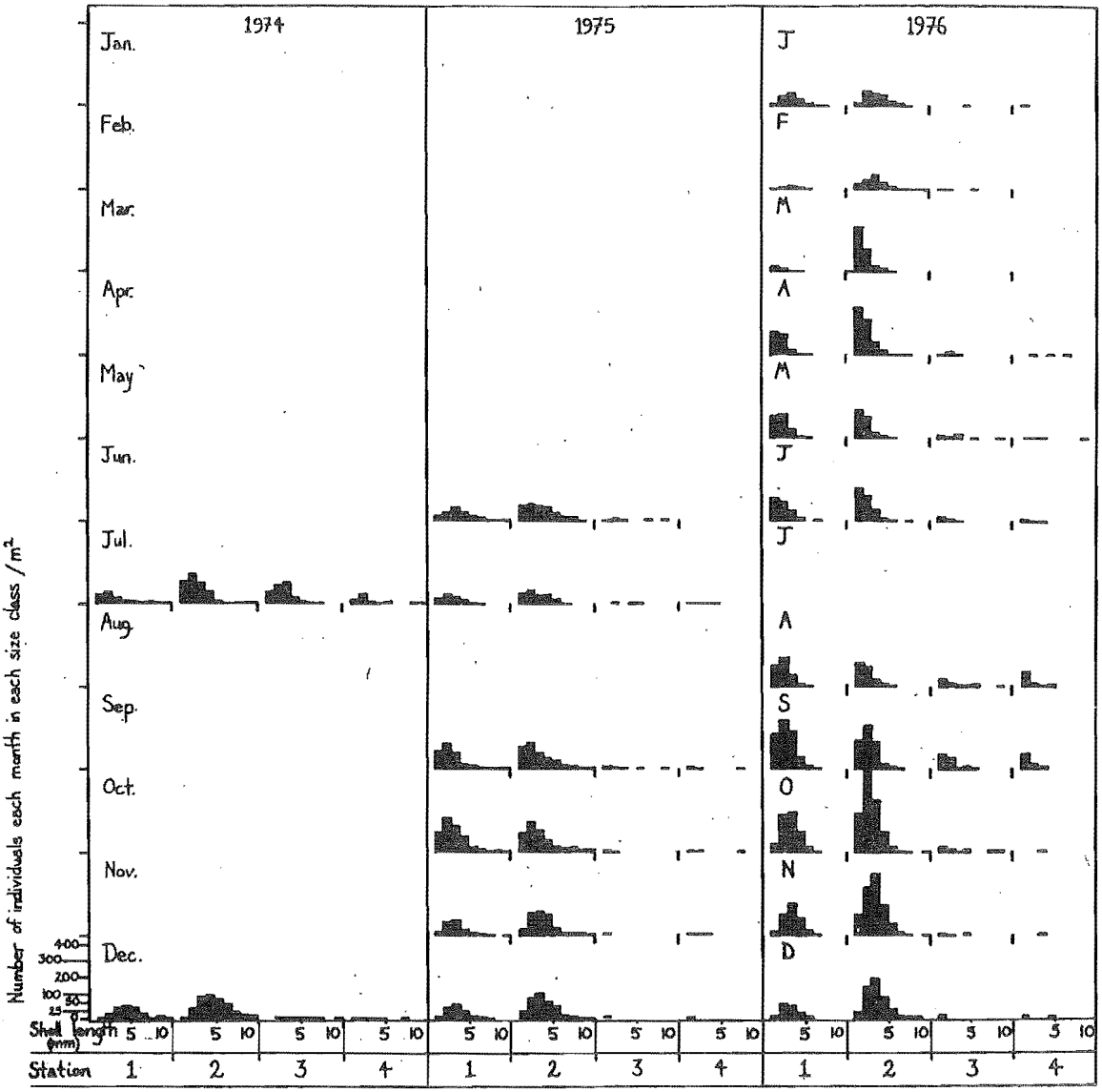


Fig. 4.3. Shell length frequency of *Amphibola crenata*, per m^2 ; less than 10mm long, at stations 1 to 4 during 1974, 1975 and 1976.

Table 4.1 Mean adult *Amphibola* density at stations 1 and 2 in Jan. 1975

Date	Stn. 1 (no/m^2)	Stn. 2 (no/m^2)
7 Jan.	4.16	1.30
13 Jan.	8.58	1.95
24 Jan.	5.2	2.99
mean of days	5.98	2.08

at stations 1 and 2 were freshly dead. Following a period of low temperatures.

The number of individuals less than 10mm at station 2 was consistently higher than at station 1 which was closest to the sewage outfall (Fig. 4.3). The seasonal trend was an increase from Jun., Jul. to Sept., Oct. 1975. with a subsequent drop in Nov., Dec. to a minimum in Jan., Feb. 1976. Station 1 reached much lower numbers in Feb. 1976 than station 2 and did not show an influx of newly settled juveniles until April, a month after station 2. After this influx, numbers fell slightly after May to a relatively stable level until Aug. when a second influx repeated the spring increase shown in 1975. An increase and subsequent decrease at station 1 occurred in Sep. and Oct. respectively, a month prior to the same changes at station 2.

The seasonal trend of small individuals at station 3 and 4 was similar to that at stations 1 and 2. Numbers dropped to a very few scattered juveniles from Oct. 1975 to Apr. 1976. A small increase in numbers occurred in May 1976 and remained stable until

Sep, when the second influx shown at stations 1 and 2 occurred.

A comparison of the coefficients of rates of change of population density of individuals less than 10mm long at stations 1 to 4 showed that although the magnitude of change was much greater at stations 1 and 2, the coefficient of rate of population change, r, as used by Gillespie (1969) in a population study of gastropods, was similar at all four stations (Table 4.II). The maximum positive values describing increases by settlement and immigration and the months in which they occurred, were 0.0444 (Apr.), 0.0379 (Apr.), 0.2688 (May), and 0.0657 (May) at stations 1 to 4 respectively. The maximum negative values describing decreases by emigration and mortality and the months in which they occurred, were -0.0524 (Feb.), -0.0281 (Jan.), -0.0452 (Jan.), and -0.1061 (Oct.) at stations 1 to 4 respectively. Stations 1 and 2 showed the same general changes of r, but there was often a month's difference in the change between the two stations.

Although shell length was more erratic, both total number and maximum shell length at stations 1 and 2 showed maximum and minimum values during Sep., Oct., Nov., and Jan., Feb., Mar., respectively (Fig. 4.4a, b). The largest animals collected during summer and winter 1974 were larger than during 1975 and 1976. Mean shell length at stations 1 and 2 was not calculated because skewness and kurtosis of the frequency curve changed during the year. The mode was plotted (Fig. 4.4c) because it is not dependent on a normal distribution. The mode gradually increased from Sep. 1975 to a maximum during Nov., Dec., Jan., with a rapid drop in Feb. 1976 to a minimum which lasted through the winter months until the large size increase was repeated in the spring.

The large size class (greater than 15mm), which occurred at

Table 4.11 Coefficients of Rates of change of Population Density

Station 1			Station 2			Station 3			Station 4		
Date	Total/m^2	r	Date	Total/m^2	r	Date	Total/m^2	r	Date	Total/m^2	r
13.06.75	135.8		13.06.75	220.0		13.06.75	6.3		13.06.75	0	
15.07	93.5	-0.0120	15.07	145.5	-0.0133	15.07	3.9	-0.0155	15.07	7.1	+0.0632
4.09	282.4	+0.0226	4.09	342.8	+0.0175	10.09	10.2	+0.0172	10.09	9.4	+0.0050
3.10	516.2	+0.0215	8.10	393.3	+0.0042	13.10	4.7	-0.0242	13.10	4.8	-0.0210
10.11	123.7	-0.0386	18.11	304.8	-0.0059	24.11	2.3	-0.0174	24.11	6.9	+0.0089
9.12	130.7	+0.0020	12.12	302.3	-0.0004	22.12	3.1	+0.0111	22.12	0.8	-0.0798
9.01.76	90.9	-0.0121	13.01.76	126.4	-0.0281	22.01.76	0.8	-0.0452	22.01.76	0.8	0
10.02	17.9	-0.0524	13.02	116.2	-0.0028	19.02	3.2	+0.0513	19.02	0	-0.0083
6.03	38.6	+0.0320	14.03	169.3	+0.0130	24.03	0	-0.0352	24.03	0	0
10.04	174.7	+0.0444	10.04	454.0	+0.0379	6.05	3.9	+0.0567	6.05	2.4	+0.0365
27.05	200.5	+0.0030	27.05	213.8	-0.0164	30.05	32.9	+0.2688	30.05	12.4	+0.0657
25.06	195.8	-0.0008	25.06	278.5	+0.0094	25.06	26.5	-0.0077	25.06	7.9	-0.0161
7.08	237.9	+0.0046	7.08	195.8	-0.0084	7.08	42.2	+0.0111	7.08	57.8	+0.0474
4.09	563.2	+0.0319	4.09	452.4	+0.0310	4.09	89.1	+0.0277	4.09	53.1	-0.0031
8.10	485.2	-0.0045	8.10	965.6	+0.0230	8.10	35.9	-0.0275	8.10	1.6	-0.1061
19.11	309.7	-0.0110	19.11	798.7	-0.0046	19.11	8.3	-0.0357	19.11	1.6	0
26.12	113.9	-0.0278	26.12	464.9	-0.0150	26.12	3.1	-0.0274	26.12	4.7	+0.0299

$$r = \frac{\log_n Nt - \log_n No}{t}$$

where Nt is the number at one sampling event, No is the number at the previou sampling event and t is the time in days between sampling events.

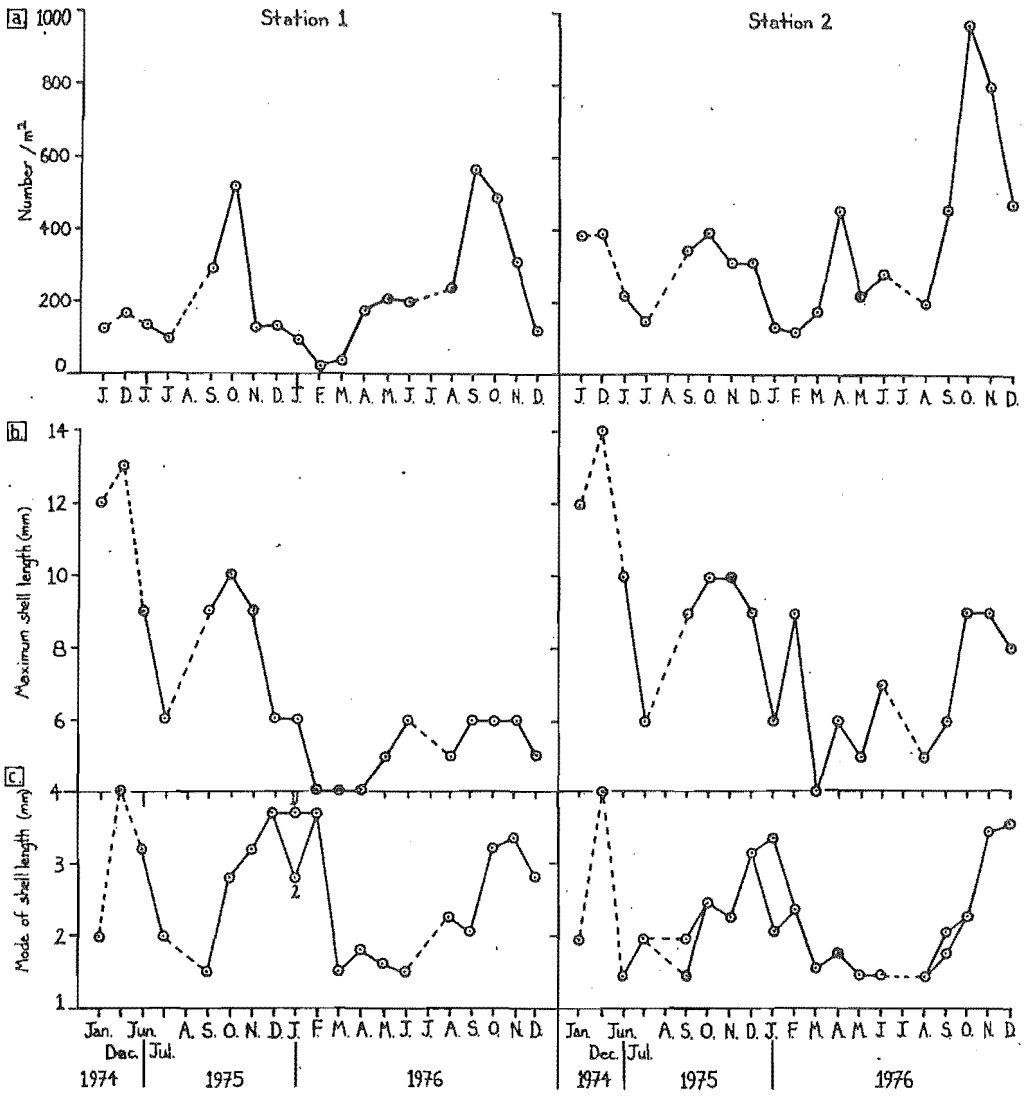


Fig. 4.4. (a) Density, (b) maximum shell length, and (c) mode of shell length of *Amphibola crenata*, less than 10mm long, at stations 1 and 2 during 1974, 1975 and 1976.

stations 3 and 4, showed very little change in size composition or total numbers during 1976 (Fig. 4.5). In 1975 there were several individuals between 10 and 18mm but this intermediate size range was almost entirely absent during 1976. The mode ranged from 21 to 23mm throughout the sampling period from Jun. 1975 to Dec. 1976 at both stations 3 and 4, and showed no seasonal trends.

The number of small individuals per m^2 at stations 3 and 4 were higher on the first sampling occasion in Jul. 1974, than subsequently in 1975 and 1976 (Fig. 4.6a). There was little change during the latter half of 1976 and the increases in Apr., May. and Aug., Sept. which occurred at stations 1 and 2, were apparent but of a lower magnitude.

The transect above station 3, sampled during Apr., May 1976 showed a zone just below H.W.L. with a population size structure quite different from that at stations 1 - 4. During the last two months, Nov., Dec. 1976 of the sampling period, this area near to H.W.L. was sampled. All size intervals 0 - 10mm, 10 - 20mm, and greater than 20mm were well represented (Fig. 4.6b). Between these two months, from Nov. to Dec. there was a drop of 57% in total numbers, the drop comprising a large decrease (76%) in the smallest size interval, partly offset by increases in the two larger size intervals.

Sieve size was shown to have a profound effect on size frequency recovery of very small newly settled individuals during Apr. 1977, at stations 1 and 2 (Fig. 4.7a). The major mode at stations 1 and 2 shifted from 1.5 to 1.1mm and from 1.5 to 0.9mm respectively. It is interesting that when the total data were plotted on a 0.1mm size interval histogram for each station

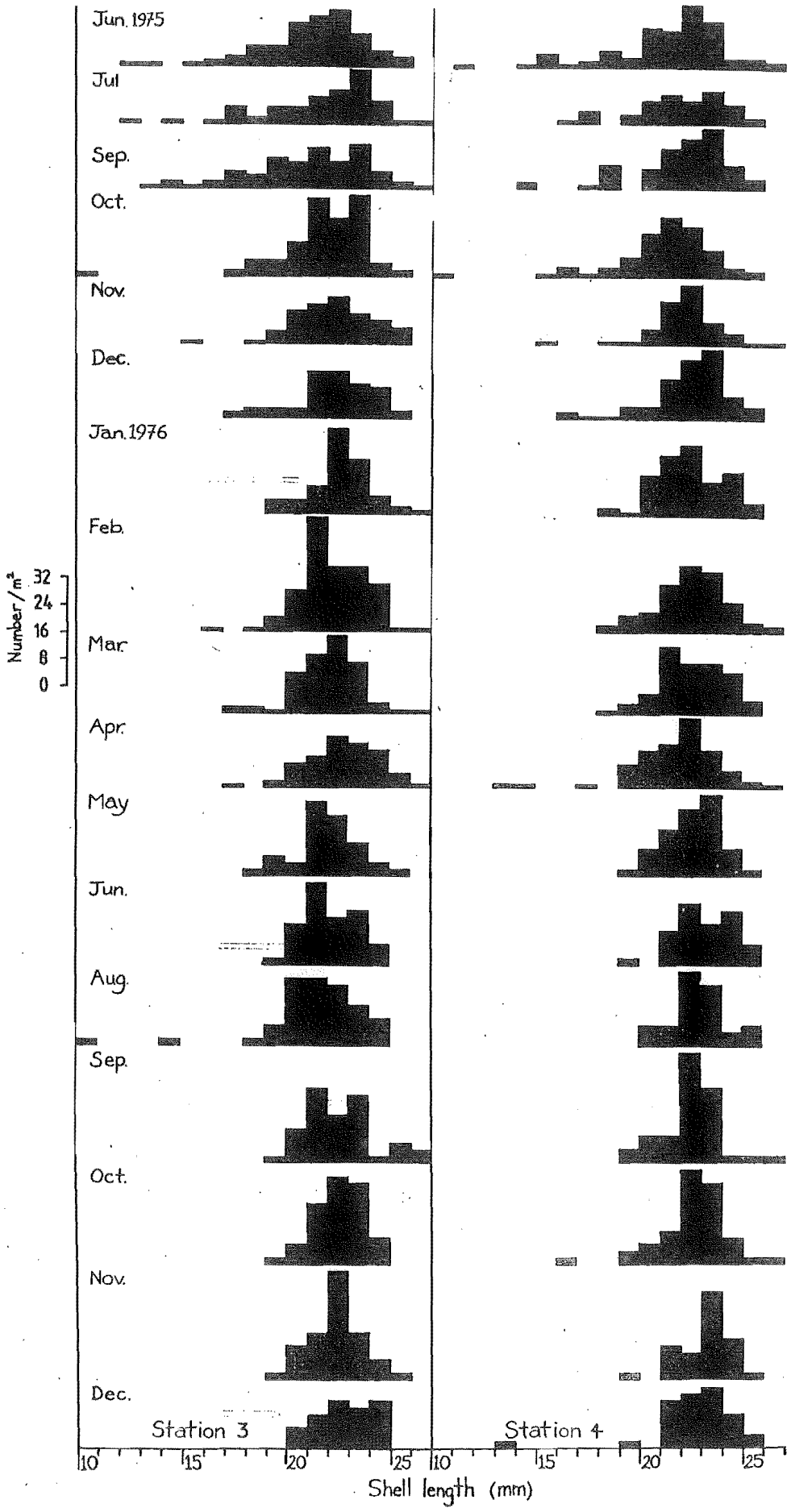


Fig. 4.5. Shell length frequency per m^2 of *Amphibola crenata*, greater than 10mm, at stations 3 and 4 in 1975 and 1976.

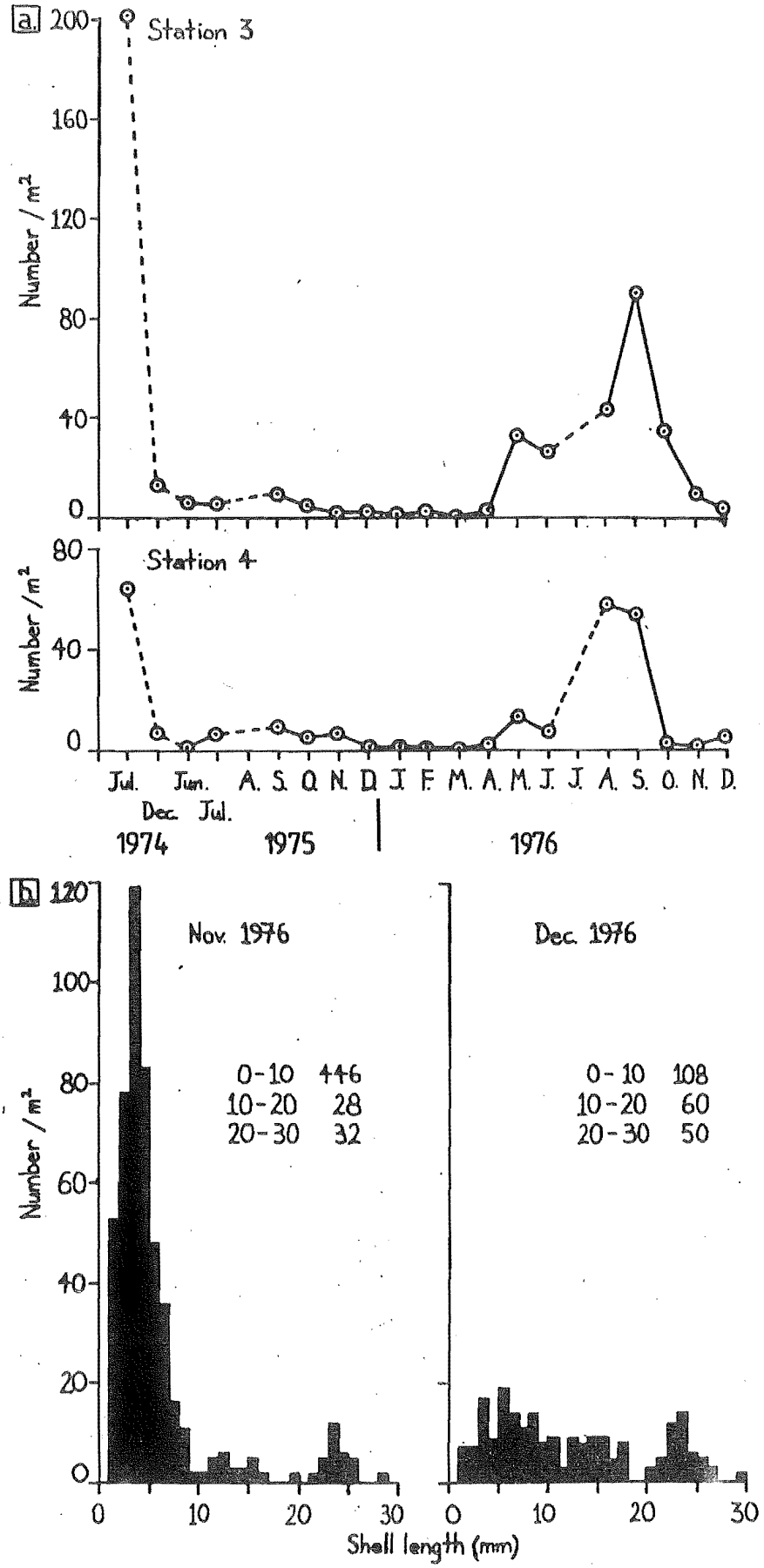


Fig. 4.6a. Density of *Amphibola crenata*, less than 10mm long, at stations 3 and 4, during 1974, 1975, and 1976,

Fig. 4.6b. Shell length frequency per m^2 of *Amphibola crenata* at H.W.L. above station 3, in Nov. and Dec. 1976.

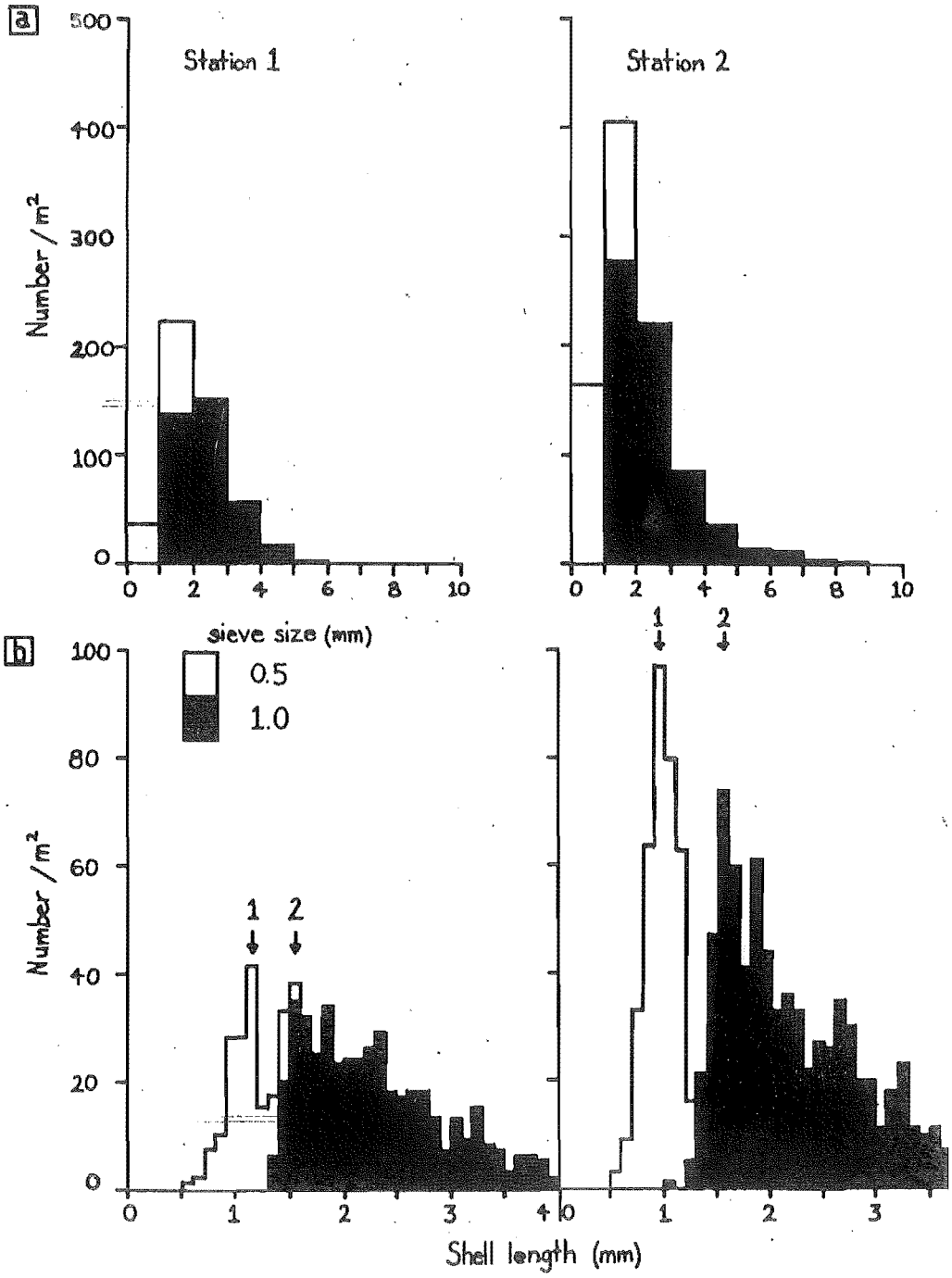


Fig. 4.7a, b. Effect of sieve size on the measurement of different shell length frequencies per m^2 of *Amphibola crenata* at stations 1 and 2 in April 1977.
a. class interval = 0.5mm, range 0 - 10mm
b. class interval = 0.1mm, range 0 - 4mm

(Fig. 4.7b), a bimodal curve resulted, with the mode derived from 1mm sieve size retaining its identity. The large number of small juveniles (less than 1.0mm), in Apr. 1977 was related back to Apr. 1976 to investigate a possible source of the large increase in numbers of individuals 2 - 3mm long, in spring 1976 (Fig. 4.8).

Wet weight was slightly greater than twice the dry weight for all 1 mm size classes less than 10mm shell length at stations 1 and 2 (Fig. 4.9) throughout the year. A plot of total weight emphasised the density pattern during 1976, showing a minimum during Feb. to Aug. with a steady increase in total weight from Sep. to Nov. and then a decrease to Dec. The mode of numbers of small individuals per shell length class increased from Sep. to Dec. with the largest increase from about 2.2mm in Oct. to about 3.5mm in Nov. 1976, but during this period the mode of weight per length class remained constant at 3mm. The 4mm weight showed a large increase in proportion to the 3mm peak showing that weight loss in this class is more than made up by growth during this part of the year.

Total weight of *Amphibola* at stations 3 and 4 showed no significant differences between stations, or between months or seasons, during the sampling period, Sep. 1975 to Dec. 1976 (Fig. 4.10a). Mean weight of individuals at both stations 3 and 4 showed an overall trend from about 2,0g in Sep. 1975 to about 2.4 in Dec. 1976 (Fig. 4.10b).

(ii) Egg laying.

The number of nidi present at all stations during the breeding season, depended on the weather, with very few nidi occurring on days with rain or a temperature below 15° C. The nidi counted would usually have been laid over the preceding 24h as beyond that period they were buried in the sediment or broken up. Nidi were first seen

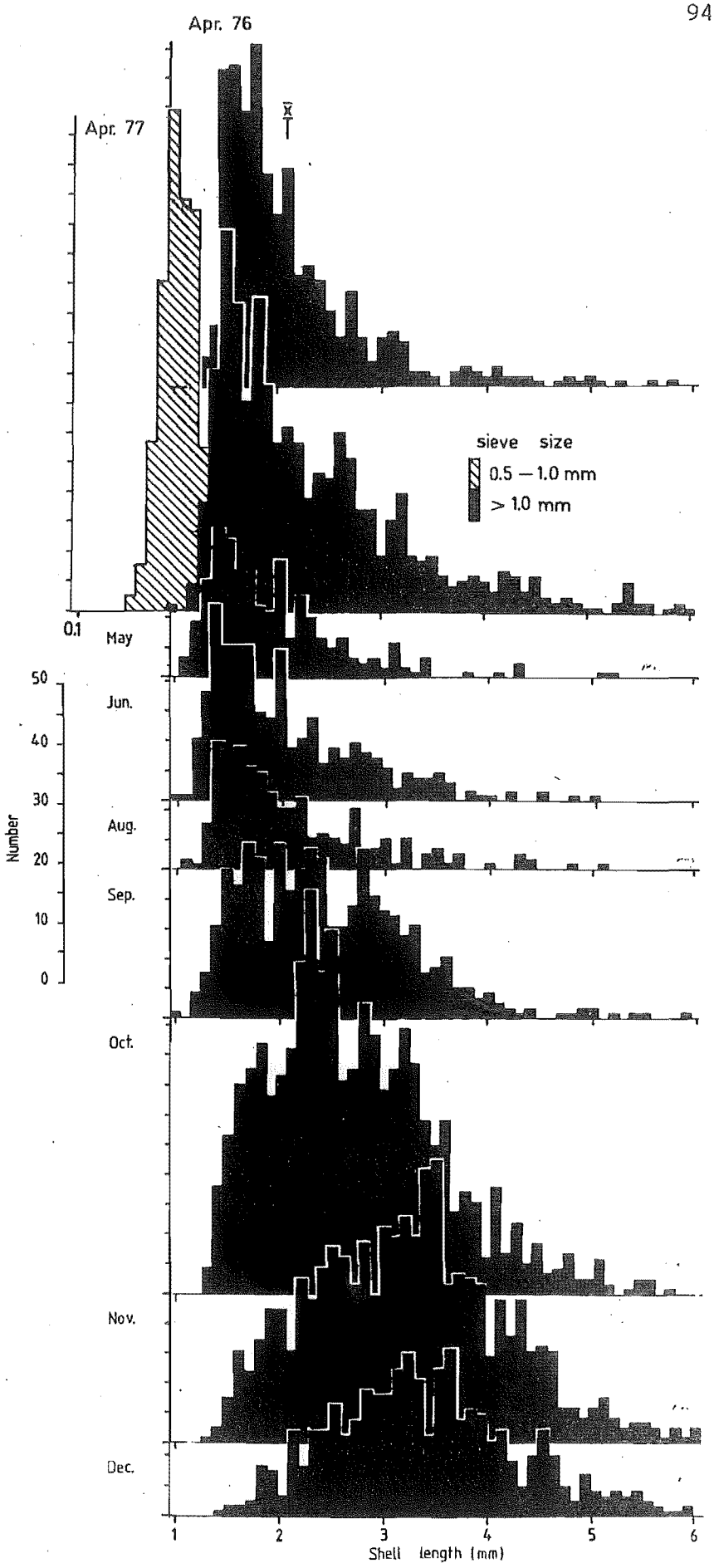


Fig. 4.8. Changes in shell length frequency per m^2 of *Amphibola crenata*, less than 10mm long, at station 2 in Apr. to Dec. 1976, compared with Apr. 1977. The effect of the smaller minimum sieve size used in Apr. 1977 is shown.

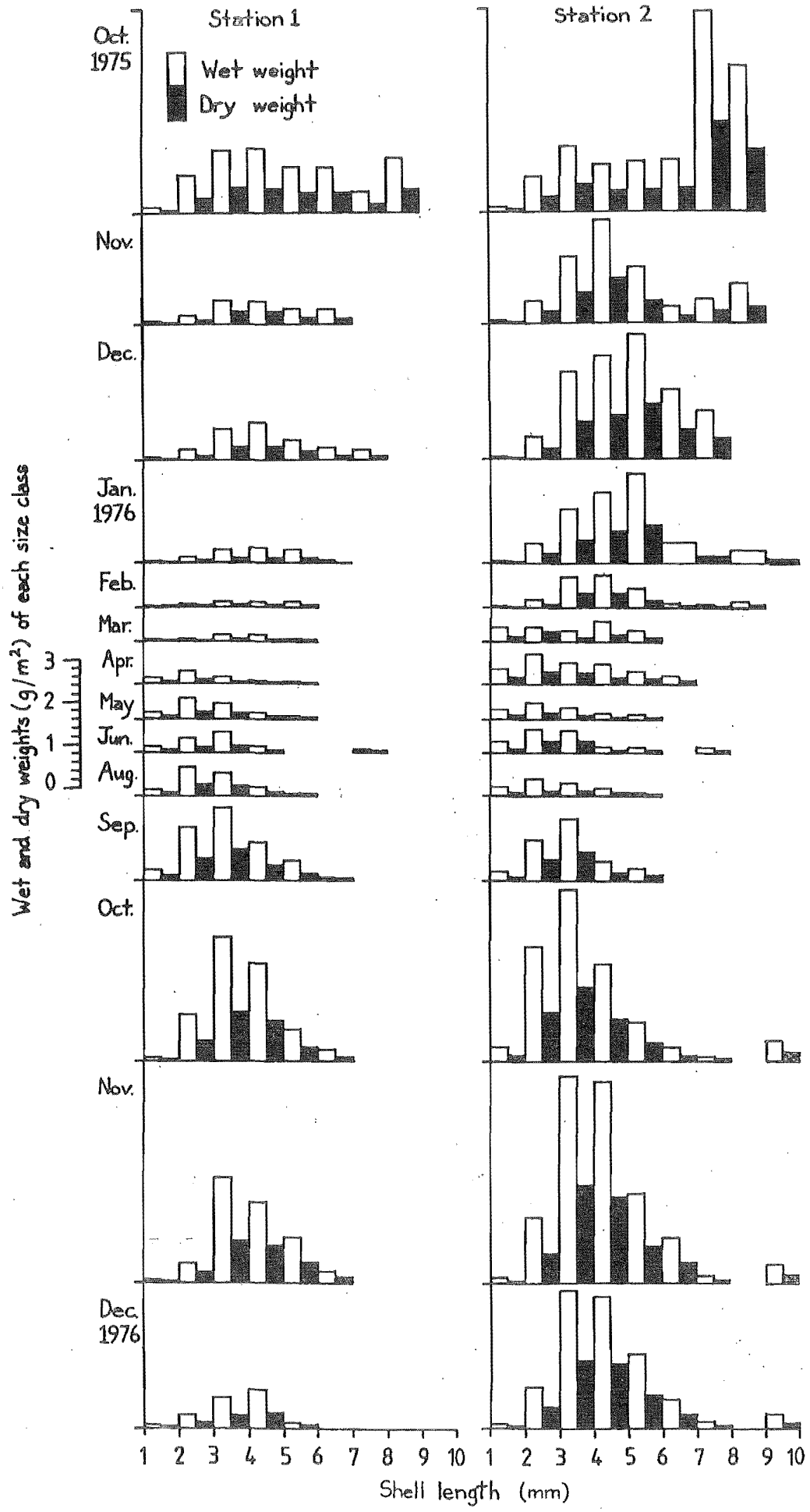


Fig. 4.9. Wet and dry weights of different shell length classes of *Amphibola crenata*, less than 10mm, at stations 1 and 2 in

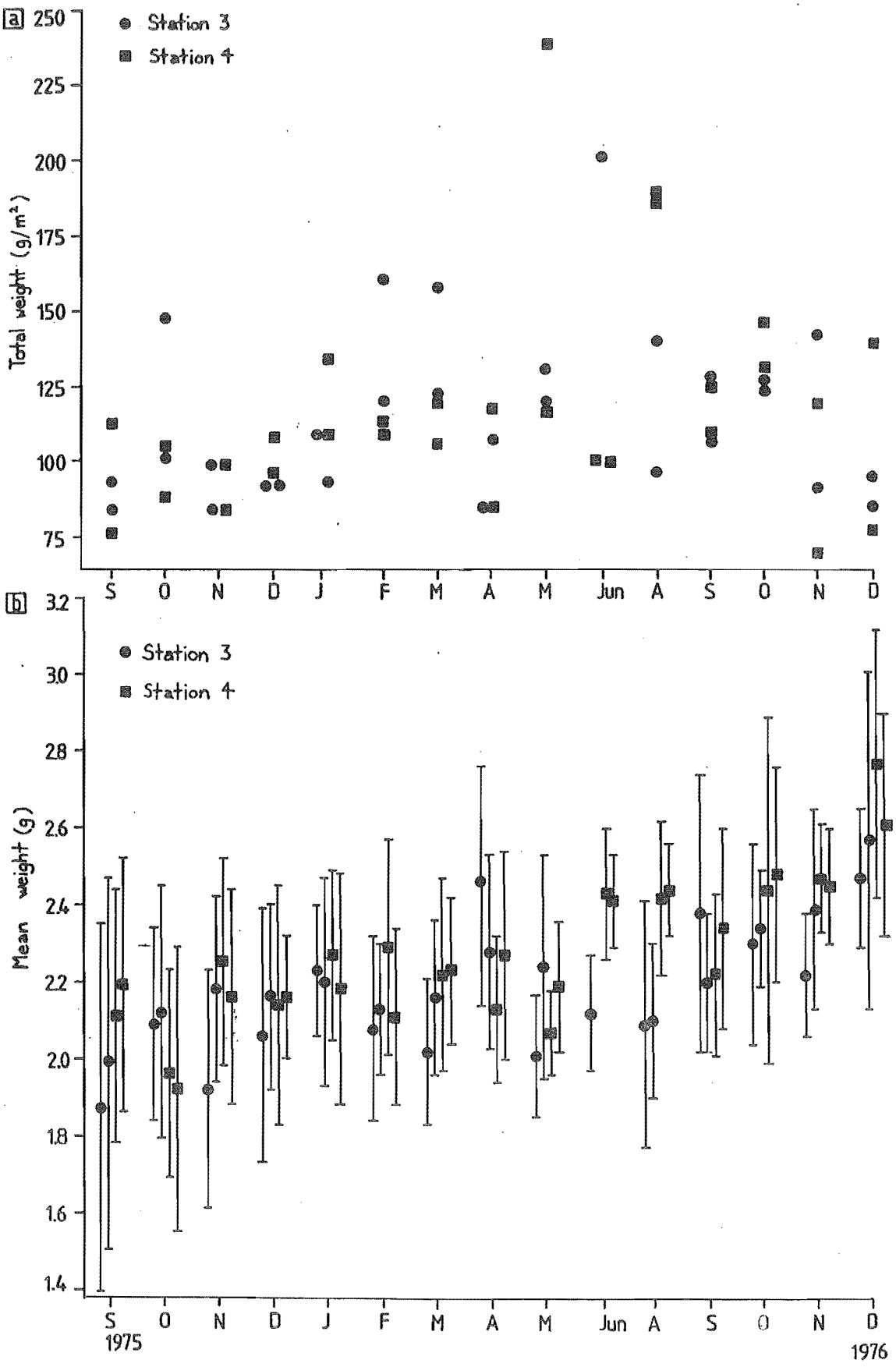


Fig.4.10 a,b. Total weight per m^2, and mean weight of *Amphibola crenata*, greater than 10mm, at stations 3 and 4, in 1975 and 1976.

at station 1 on 10 Nov. 1975 and the highest number of nidi per *Amphibola* (0.64) also occurred at station 1 (Table 4.III). The *Amphibola* at station 1 were all large and therefore it would be expected that they were all breeding adults. Station 2 has a slightly higher nidi per *Amphibola* count than stations 3 and 4 which were equivalent. The highest density of nidi was at station 3 with 14.62 per m. The nidi at stations 3 and 4 were larger where the sediment was raised and therefore better drained.

Table 4.III Nidus density at stations 1 to 4, Jan., Feb. 1975

Date	AT_1	MT_1	Stn 1 n/m^2	Stn 1 n/A	Stn 2 n/m	Stn 2 n/A	AT_3	MT_3	Stn 3 n/m2	Stn 3 n/A	Stn 4 n/m	Stn 4 n/A
13.1.75	22	27.5	1.56	0.18			23.5	27				
21.1.75	21	24	9.62	1.85	1.17	0.39	20.5	24.5	42.25	0.55	37.31	0.67
17.11.75	16	17	7.02	0.69	2.34	0.25	16.	18	0.78	0.01	0	0
18.11.75	18	19	7.80	0.27	3.12	0.44	19	21.50	42.12	0.52	12.48	0.25
19.11.75	20	21.5	15.60	0.74	0.78	0.08	19.5	22	12.48	0.17	10.14	0.16
20.11.75	22	22	3.12	0.29	4.68	0.55	23	23	0.78	0.01	0.78	0.01
26.11.75	19	21	2.34	0.43	not measured		19	22.5	3.90	0.07	14.82	0.25
mean			6.72	0.64	2.02	0.29			14.62	0.19	10.79	0.19

Where AT_1 MT_1 is air temp and mud temp respectively at station 1.

AT_3 MT_3 is air temp and mud temp respectively at station 3.

n/m is the number of nidi per m^2.

n/A is the number of nidi per adult *Amphibola*

(iii) Comparison of size-frequencies at other areas of the estuary.

Shell length and density distribution of *Amphibola* along transects from H.W.L. to L.W.L. were examined in relation to general sediment size patterns (Fig. 4.11). All size classes were found at transects 2 to 20 which covered almost all of the ecological range of *Amphibola* in the estuary. In the lower reaches of the rivers, above the estuary flats, all sizes occurred in most samples and the distribution extended for similar distances along the two rivers. Density was highest in areas of predominantly small individuals and reached a maximum on the western estuary slopes at transects 11 to 14 and 17 in the area and upstream (Avon R.) of the sewage outflows. The two size intervals less than 10 mm occurred throughout the ecological range from transects 1 to 22. They comprised a decreasing proportion below M.W.L. and very rarely occurred at L.W.L. or where sediment was less than 5% silt and clay. In the area closest to the sewage outflow other sizes were rare, but in most parts of the estuary the sample contained a proportion of all size classes.

Intermediate sized individuals (11 - 20mm) occurred predominantly in the lower Heathcote R. and estuary flats at transects 6 to 9 and transect 19. These transects covered a transition area where particle size and salinity were intermediate between the lower estuary and further up the rivers.

Amphibola over 20mm long occurred at all tidal levels, and comprised the largest proportion of samples from the central estuary where silt-clay content was less than 20% and where salinity was highest.

The shape of the size frequency distribution (Fig. 4.12) showed a trend towards decreasing numbers of the smaller sizes and an

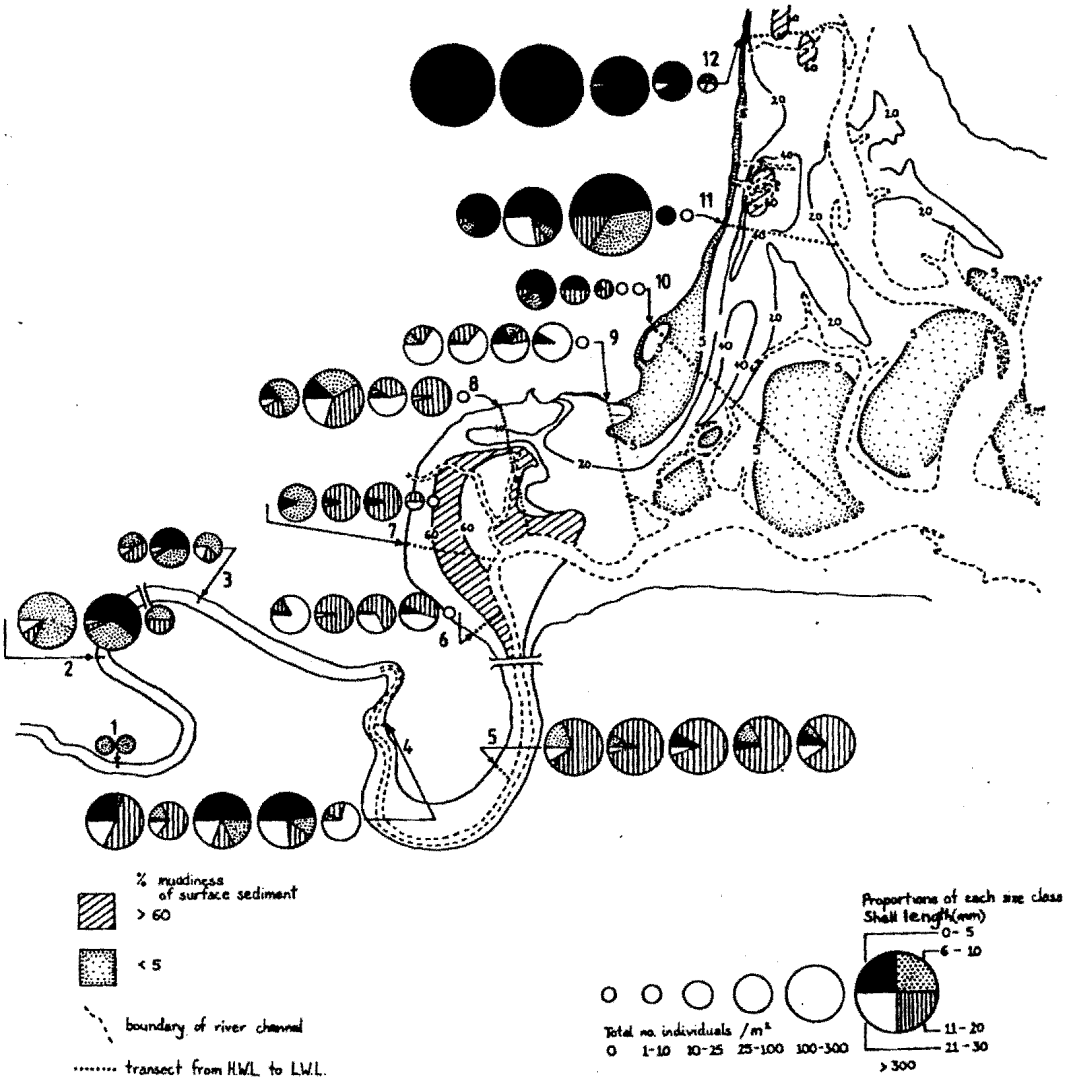


Fig. 4.11. Facing pages. Shell length frequency per m^2 and total population density of *Amphibola crenata* at sites along transects from H.W.L. to L.W.L., across the Avon-Heathcote Estuary. The transects cover the ecological range of *A. crenata* from its furthest penetration up the Avon and Heathcote Rivers to its limit near the mouth of the Estuary. The muddiness of the surface sediment is shown, after Macpherson (1977).

Note the difference in scale of the land, and the pie diagrams, on the two pages.

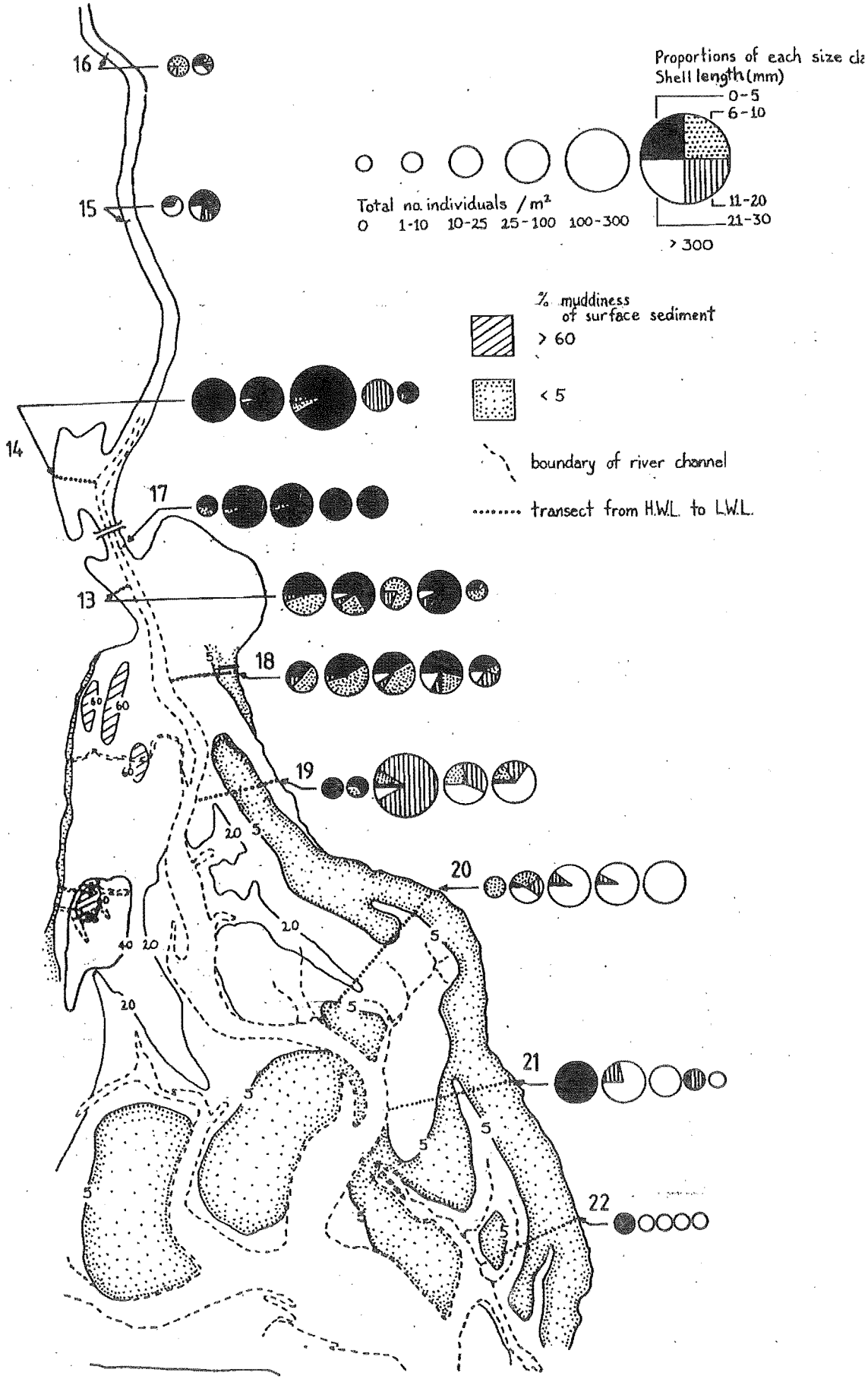


Fig.4.11 Continued from facing page

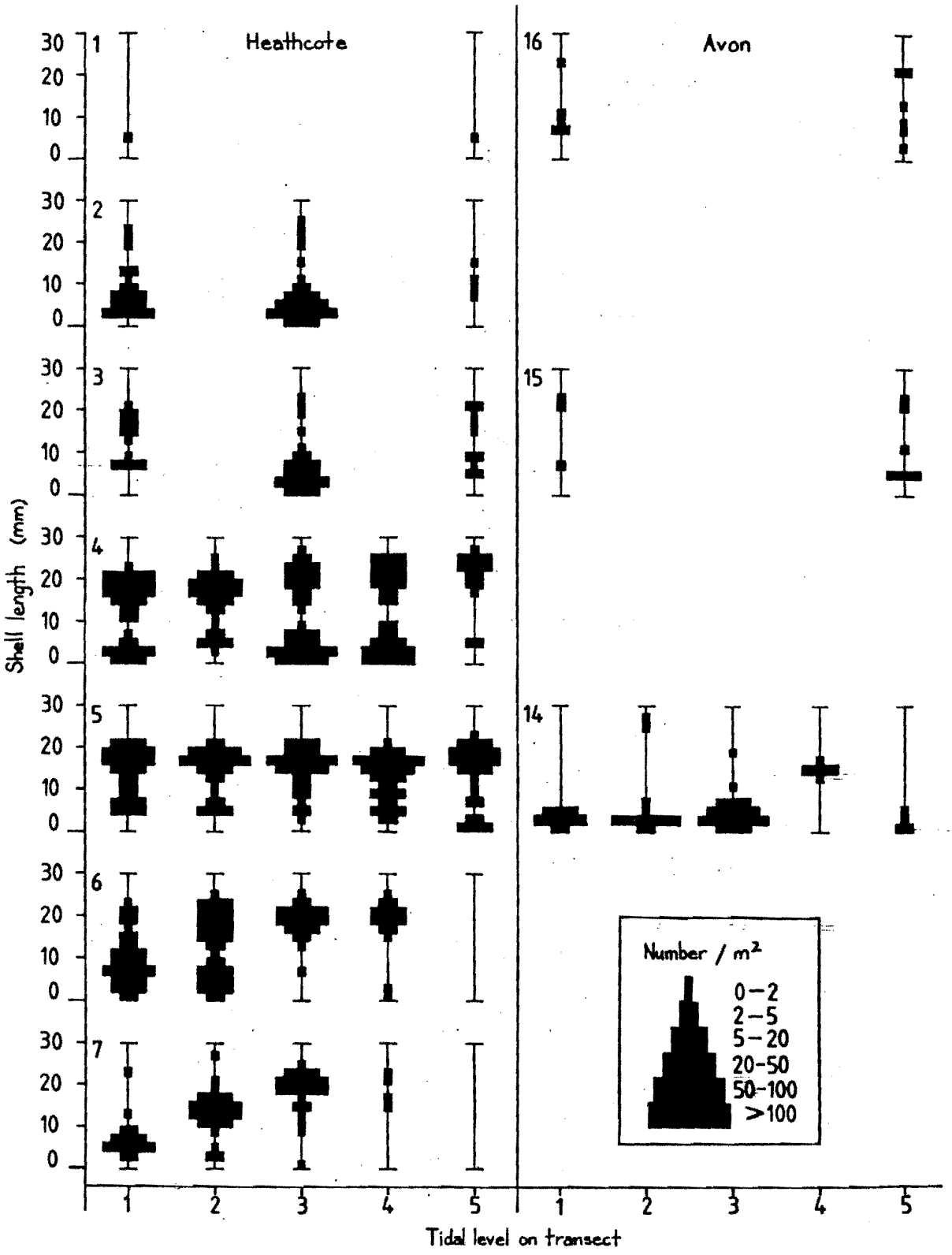


Fig. 4.12. Shell length frequency per m^2 of *Amphibola crenata* along transects from H.W.L. to L.W.L. across the Avon-Heathcote Estuary. The transects are arranged to compare sites at similar distances from the estuary mouth.

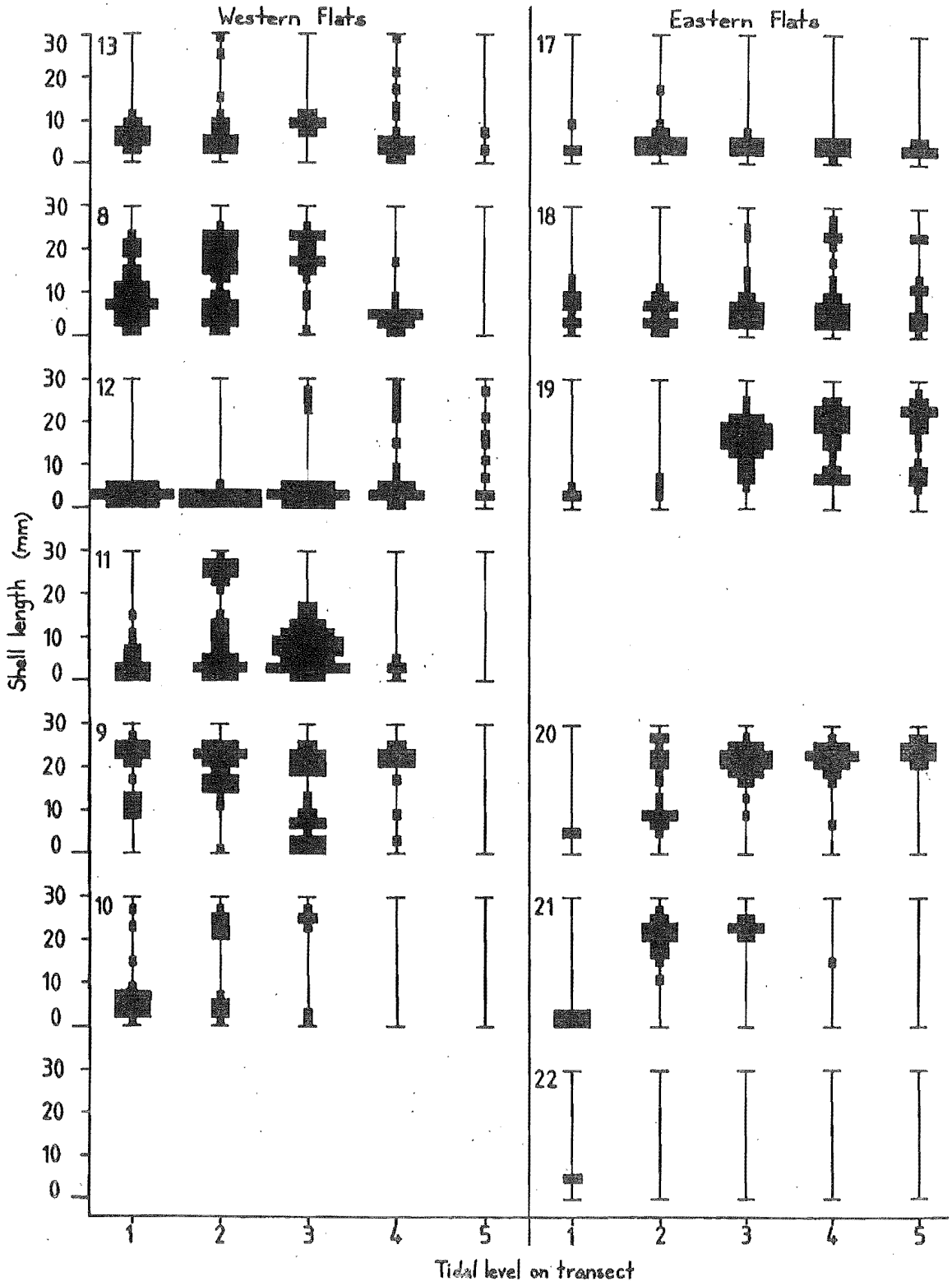


Fig. 4.12. Continued

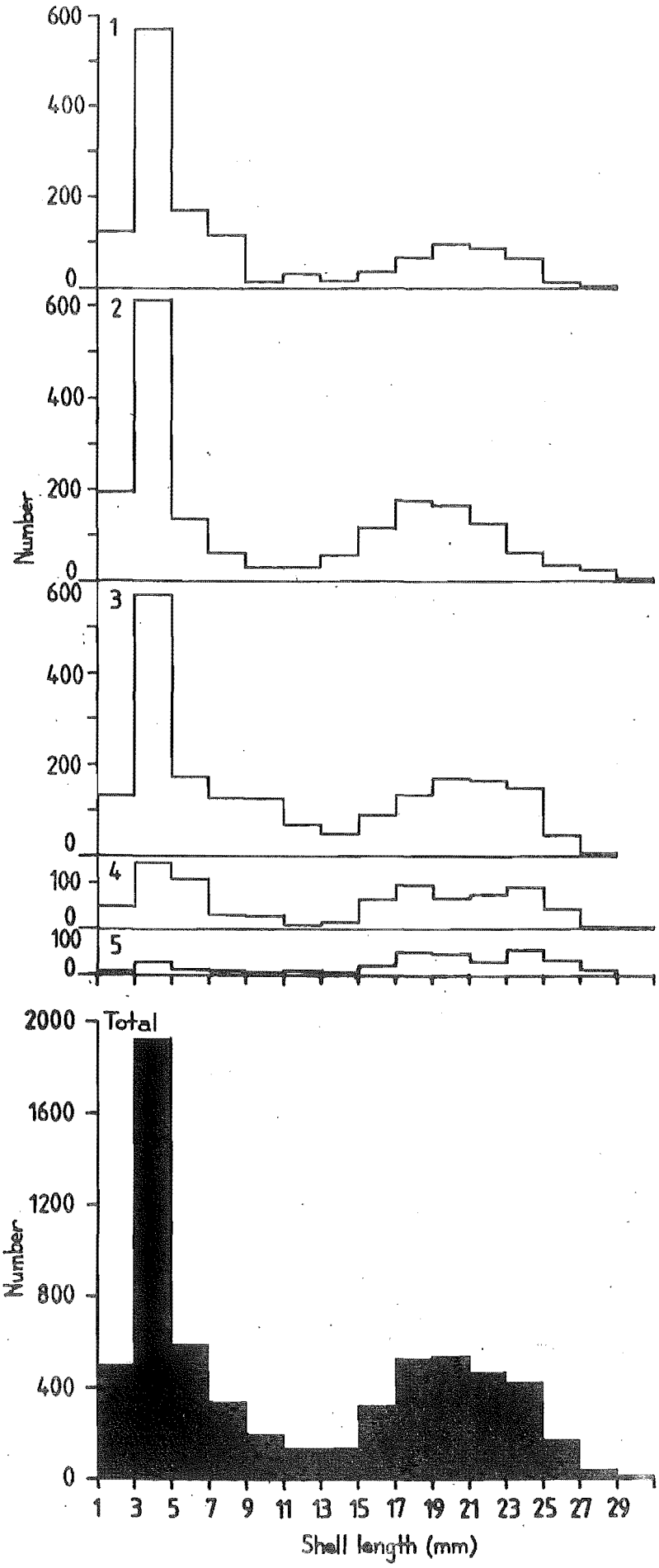
increase in the mode of the larger sizes from H.W.L. to L.W.L. A comparison of transects at similar distances from the estuary mouth showed no apparent relationship between size distribution and position along the estuary river profile. Most samples showed a distinct single or bimodal pattern and examples of both occurred at all tidal levels. In no samples did a single mode occur in the 10 - 15mm size intervals.

The size frequency distribution was summarised in a single histogram for each tidal level (Fig. 4.13), and for the total survey excluding transects 1, 2, 3, 15, 16, at the limits of the range along the rivers, where five tidal levels were not represented. The magnitude and shape of the histogram was similar at tidal levels 1 to 4, and all tidal levels showed a bimodal distribution. At levels 4 and 5 the second mode was divided into two further modes. The proportion of the sample under the first mode (Table 4.IV) fell from 66% at H.W.L. to 22% at L.W.L. The number of individuals in the first mode was highest at level 2 but the highest proportion was at level 1.

Table 4.IV. Percentage of sample less than 10mm long at each tidal level in the total estuary survey

Tidal level	Total Number (0-30mm)	% less than 10mm
1 (H.W.L.)	1494	66
2	1821	57
3	1987	56
4	826	44
5 (L.W.L.)	322	22

Fig. 4.13. Shell length frequency of *Amphibola crenata* at each tidal level, or exposure level, for all of the transects except those where five tidal levels were not represented. Tidal level 1 at top is H.W.L., while 5 is L.W.L. The number of *A. crenata* at each tidal level refer to equivalent total areas.


600
400
200
0
1
600
400
200
0
2
Number
600
400
200
0
3
100
0
4
100
0
5
2000
1600
1200
800
400
0
Total
Number
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Shell length (mm)

The second mode occurred at shell length 17 - 20mm, while the third, at tidal levels 4 and 5 was at 23 - 26mm. Levels 2 and 3 had the highest number of animals, with level 3 (M.W.L.) comprising 31% of the total for all five levels.

When total dry weight (including shell weight) was plotted for the transects across the estuary (Fig. 4.14) it was apparent that total weight of *Amphibola* per m^2 was greatest at transects 4 to 6 where individuals 10 - 19mm made the largest contribution, and transects 19 and 20 where animals greater than 20mm long made a large contribution. Overall, the weight of animals less than 10mm was relatively small compared with the two larger size intervals which each made up similar proportions of the total weight. The weight of *Amphibola* was similar at all five tidal levels as the increasing proportion of larger individuals towards L.W.L. compensated for decreasing density. The highest weights per m^2 occurred at transect 6, level 1 (198.0g) and transect 20, level 3 (194.7g). The largest weight per m^2 was not related to the highest organic content although the highest densities of small individuals occurred on this area close to the sewage outfalls.

A transect from H.W.L. above station 1 showed a zone down to 25m below H.W.L. where no *Amphibola* occurred (Fig. 4.15). A relatively constant size frequency was found along the transect from below this point, to below station 1 about 100m below H.W.L. This size frequency was similar to that described for stations 1 and 2. Below 80m the silt-clay and moisture content of the sediment increased, and a decrease in the number of small *Amphibola* was accompanied by a slight increase in the number of larger individuals.

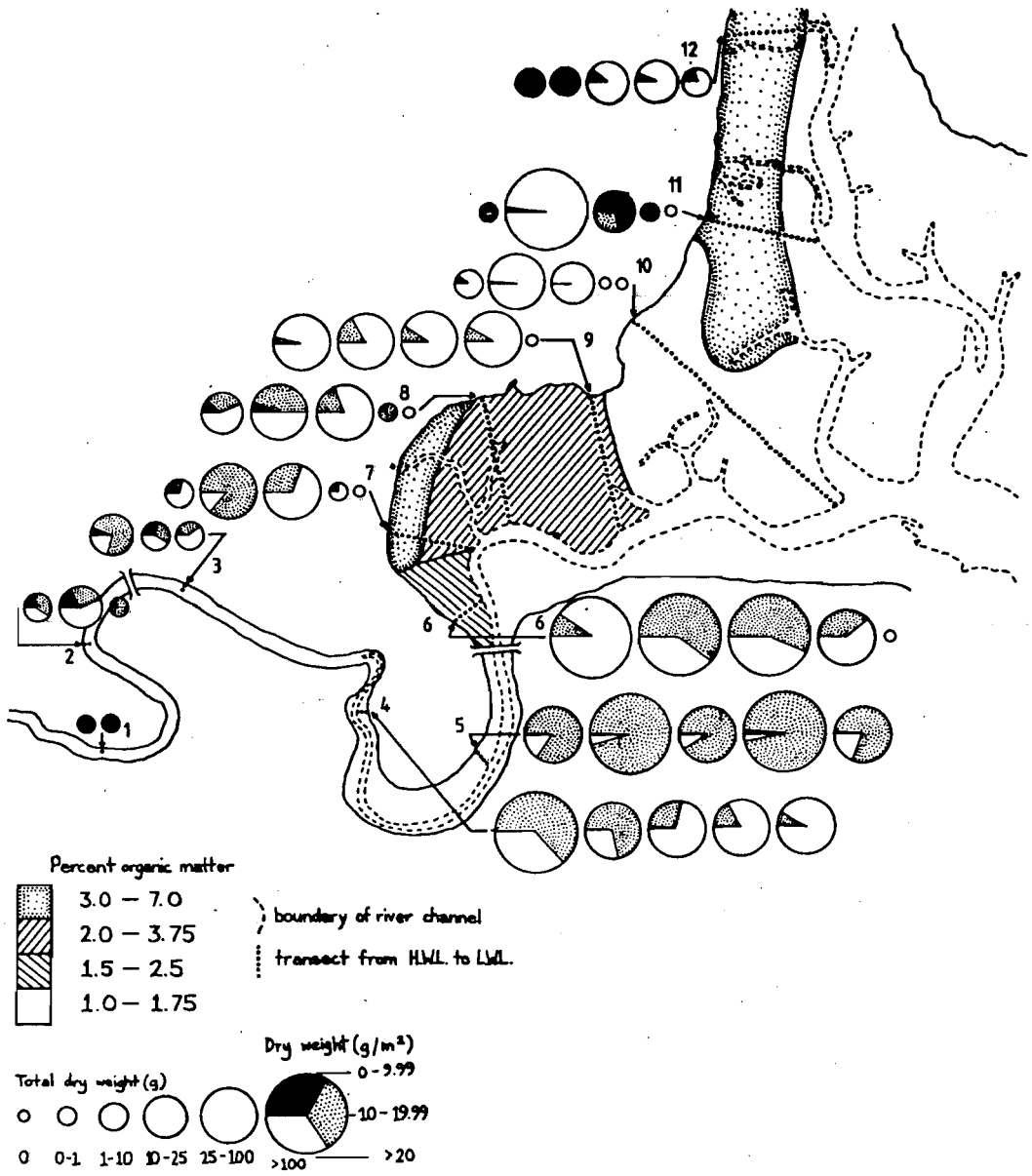


Fig. 4.14. Dry weight frequency per m^2 and total weight of *Amphibola crenata* at sites along transects from H.W.L. to L.W.L. across the Avon-Heathcote Estuary. The organic content (percentage dry weight) of the surface sediment is shown, after Knox and Kilner (1973).

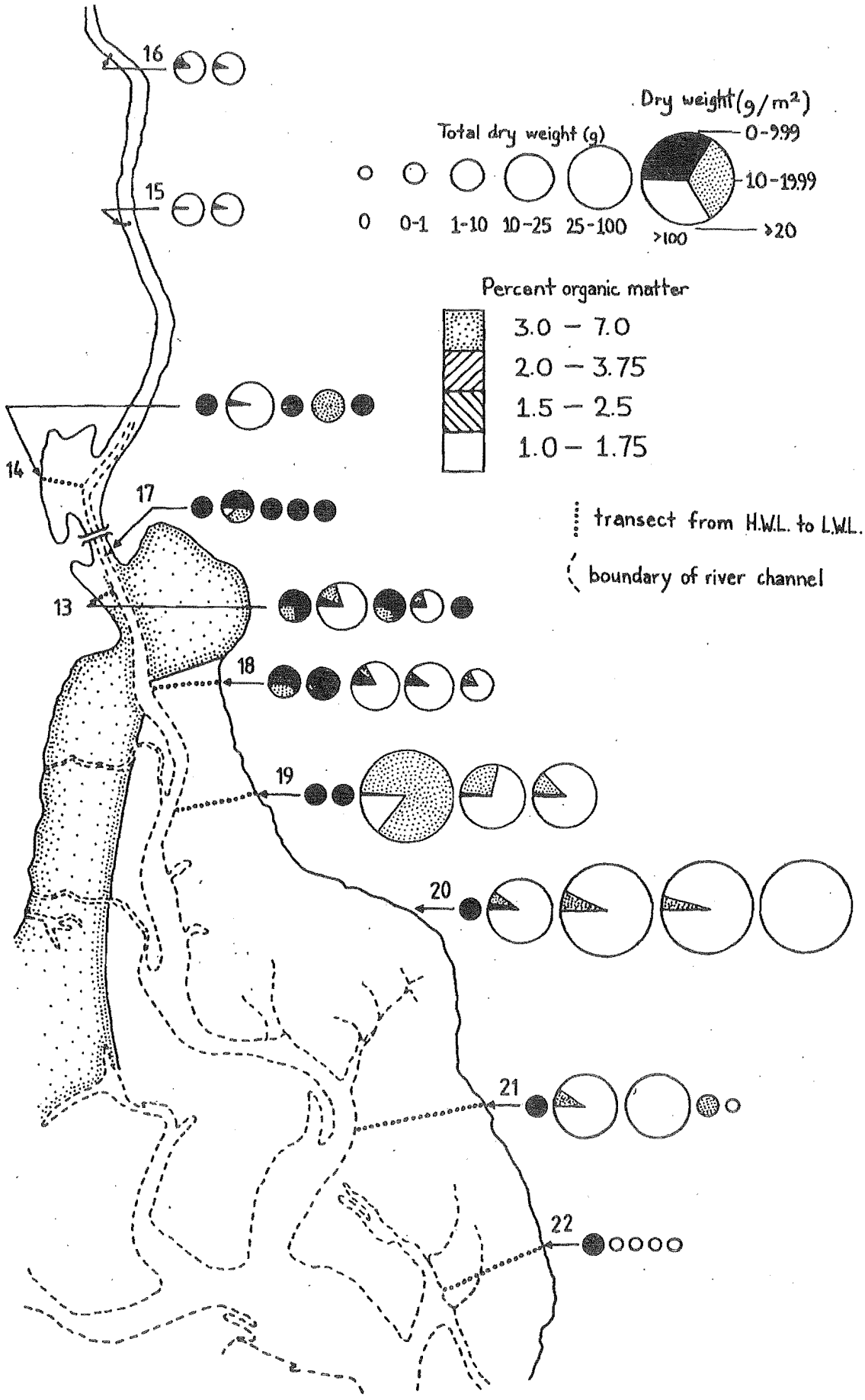


Fig. 4.14 Continued.

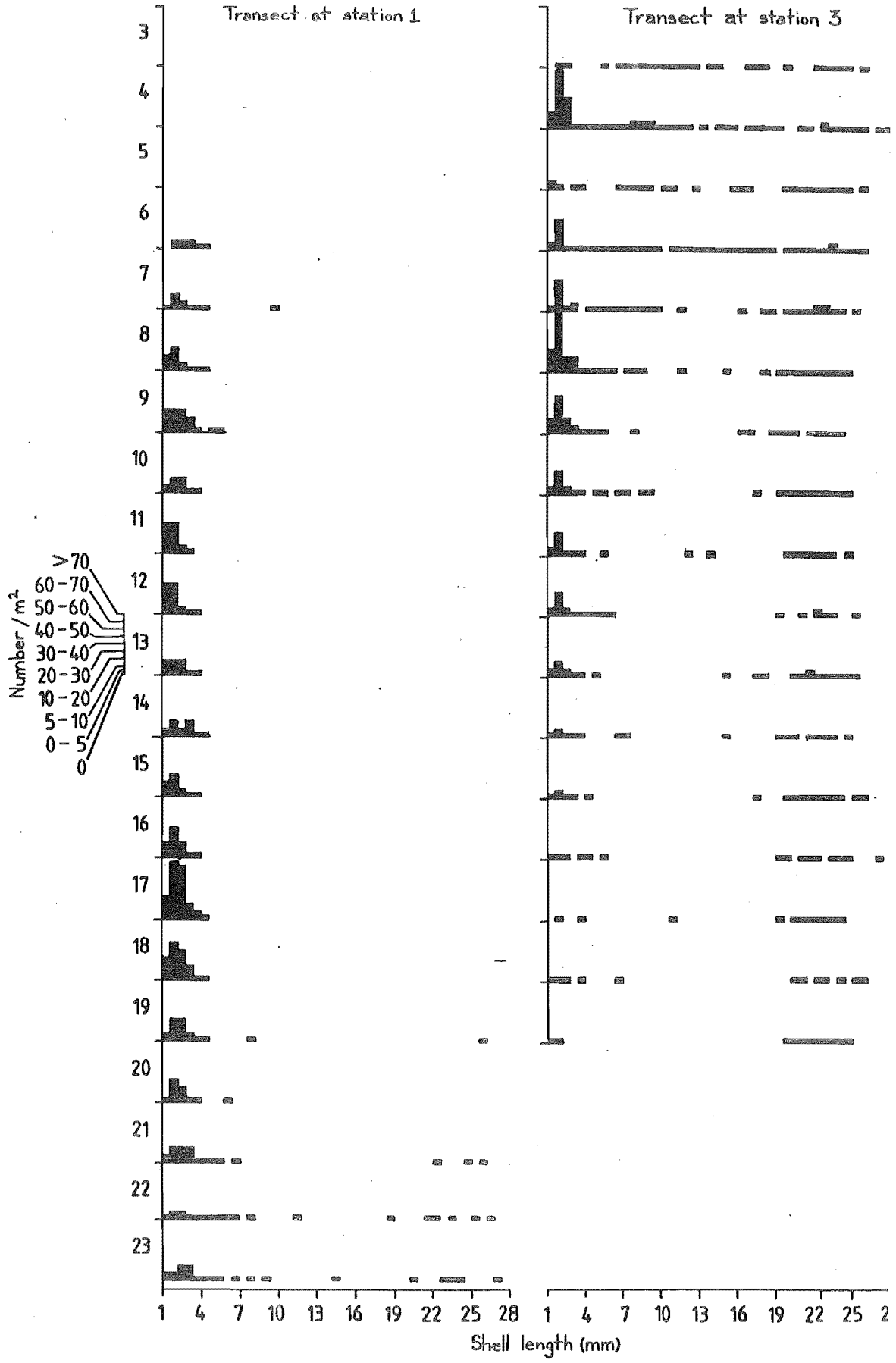


Fig. 4.15. Shell length frequency per m^2 of *Amphibola crenata* at sites along transects from H.W.L., normal to the shore, above stations 1 and 3, in Apr., May 1976.

A transect from H.W.L. above station 3, down to station 3, covering about 200m (Fig. 4.15) showed a relatively constant size frequency down to 100m below H.W.L. This frequency comprised a high proportion of small individuals and a range of larger animals up to 25mm long. Individuals 10 - 18mm long decreased over the first 80m and were almost entirely absent below this. From 100m below H.W.L., down to station 3, the number of small individuals decreased from as high as at station 1, to very few, while the number of individuals of larger sizes, above 19mm long, remained relatively constant.

(iv) Shell sculpture.

Sculpture of shells from stations 3 and 4 remained relatively constant throughout the sampling period (Fig. 4.16). About 25 - 50% of the population at both stations 3 and 4 had no sculpture. The proportions of each sculpture indice and the mean sculpture index showed no marked seasonal pattern and were generally similar at each station on any month. A slight increase in mean sculpture index was apparent during Sep. to Dec. in both years.

Shell sculpture was compared at all transects (Fig. 4.17) and no clear trend in respect of tidal level or distance from the estuary mouth was shown. Heavy sculpture did not occur up the Avon and Heathcote Rivers above transect 4 and transect 18, or at transects 10,20 and 21 closest to the estuary mouth. Heavy sculpture was never found at

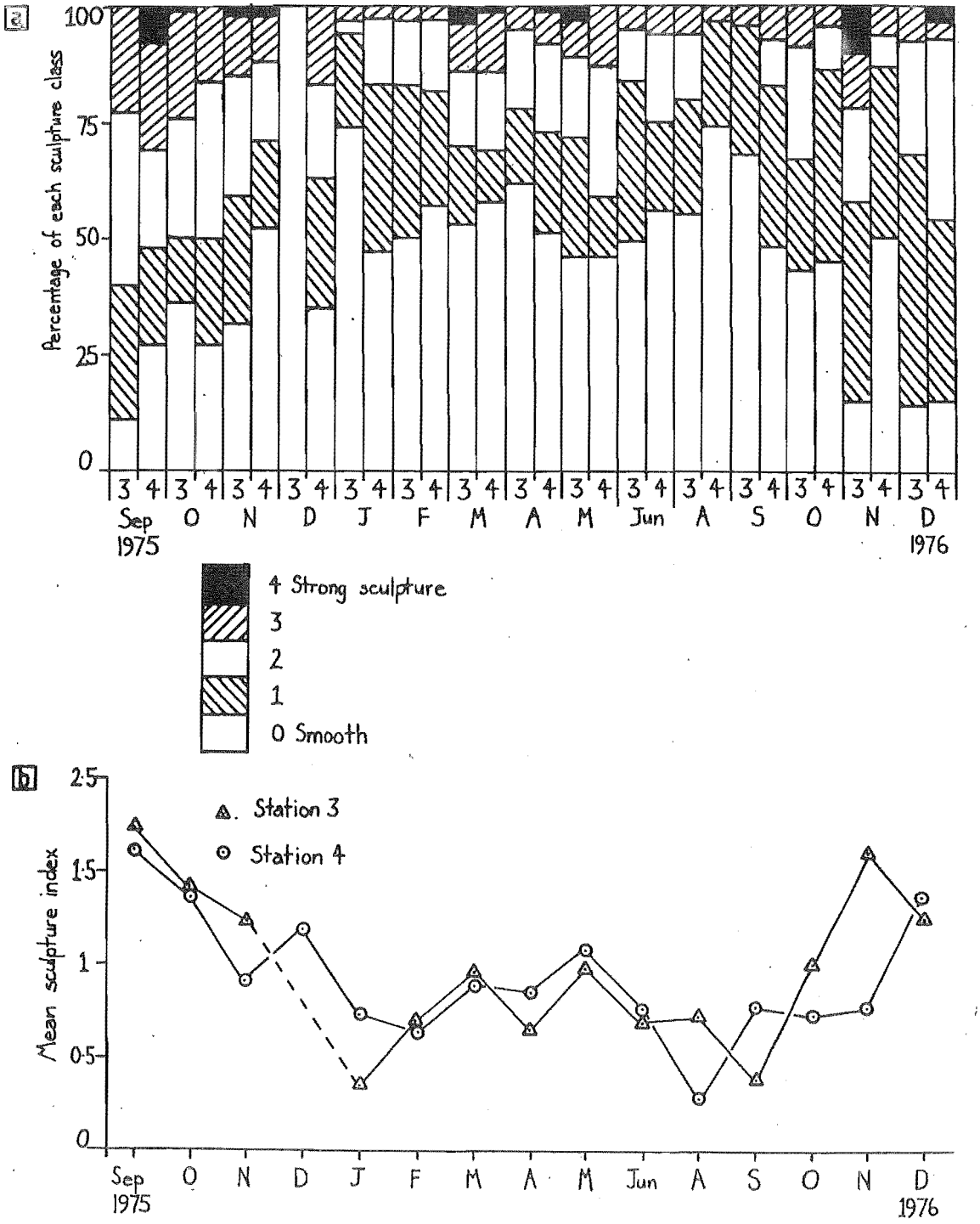


Fig. 4.16. Shell sculpture indices and mean shell sculpture index of *Amphibola crenata*, greater than 10mm, at stations 3 and 4, in 1975 and 1976.

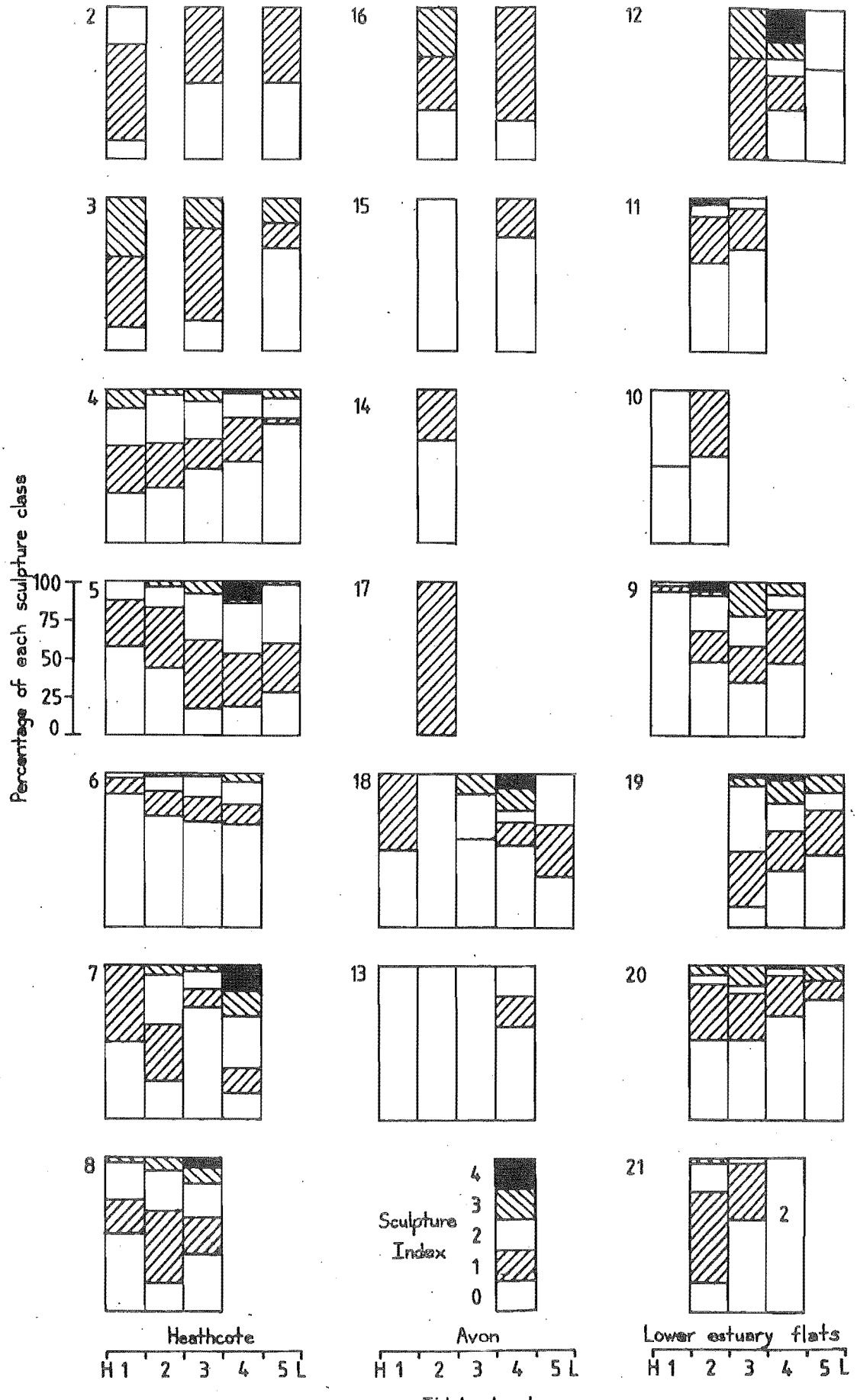


Fig. 4.17. Shell sculpture indices of *Amphibola crenata* at sites along transects from H.W.L. to L.W.L. across the Avon-Heathcote Estuary. The transects are numbered according to Fig. 4.12 and arranged to compare sites at similar distances from the estuary mouth.

H.W.L. or L.W.L. but occurred at half the transects on one of the levels 2, 3 or 4. The largest proportion of individuals with heavy sculpture occurred along transects 5, 7, 12 and 18 at level 4. These transects represented two equivalent areas where the estuarine flats narrowed into the lower reaches of the Avon and Heathcote Rivers. The proportion of smooth or unsculptured individuals was greatest, however, at transects 6, 13, 18 and 20 which covered the same area as the heavy sculpture as well as closer to the estuary mouth. Most samples with a sufficient number of animals larger than 5mm showed a representation of the whole range of the sculptural index, although smooth individuals (0) comprised the largest proportion over the estuary.

2. Sediment Preference

(i) Sediment preference under laboratory conditions.

Table 4.V Preference for sediment from stations 1 and 3

Expt. No.	No. of animals	No. of trials	$\bar{X}$ (mm)	Range (mm)	"Preference" stn 1 or 3	χ^2	p
(a)	40	10	8.10	6.2 - 15.0	1	84.88	<0.001
	8	10	24.76	22.7 - 26.6	1	5.0	0.025
(b)	24	10	17.94	14.3 - 20.7	3	0.15	0.7
(c)	12	10	23.05	17.7 - 27.1	3	6.53	0.01

Small animals showed a significant preference for the sediment from station 1, medium sized animals ($\bar{X}$ = 17.94) showed no preference, while a group covering the size range found at station 3 ($\bar{X}$ = 23.05) showed a slight preference for station 3 sediment and a group of large animals ($\bar{X}$ = 24.76) similar to those which occurred sparsely at station 1 showed a slight preference for station 1 sediment (Table 4.V).

(ii) Sediment ingestion in the natural habitat.

There was as much variation in sediment from two replicate samples of small *Amphibola* from station 2 as between the different size classes at stations 2, 3 and H.W.L. above station 3. The proportions of sediment particle size classes egested by the animals were similar to that sampled at the sediment surface. There was a higher proportion of fine particles less than 0.05mm at stations 1 and 2 than was recorded during sediment particle size determination by sieving, but the sampling for these experiments removed a much thinner surface section of sediment.

(iii) Sediment ingestion under controlled habitat conditions.

Animals from cages at stations 1 and 2 egested much more fine material than at stations 3 and 4. This high proportion of silt material resulted from the excavation necessary to place the cage in position. This artifact limited the value of the experiment to a comparison of size classes within each station. All size classes were capable of ingesting the sediment present and no difference in egested sediment particle size was found between animal size classes from the cage at each station.

IV DISCUSSION

1. Seasonal changes in size-frequency distribution at stations 1 to 4

Polymodal size frequency distributions, representing different year classes, have been described for populations of molluscs with a restricted reproductive period and a relatively uniform growth rate (e.g. Franz 1971, Hughes 1970). The results of the present study showed an essentially bimodal frequency over the total estuary during Apr. to Jun. 1975.

Nidus formation in the Avon-Heathcote Estuary in 1975 and 1976 was noted over a period of five months each year, from early Nov. to Mar. This observation agreed with Farnie (1924) who described the beginning of nidus formation in early Nov. at Otago, but contrasted with a much longer reproductive season from Aug. to mid-May, with a maximum in Apr., observed by Briggs (1972) in Northland. Station 1 had the highest nidus count per number of adults showing that nidus formation was not limited by water quality or reduced mating contact between individuals in a sparse adult population. This high count may have been because all the large *Amphibola* at station 1 were mature adults. Nidus formation appeared to reach a maximum during Feb., although this was affected by weather conditions. Each breeding individual produced about one nidus each 5 days, and Briggs (1972) estimated a mean number of 7,500 eggs per nidus.

The first influx of juveniles achieving 1mm sieve recovery size appeared at stations 1 to 4 in Mar., and Apr. These presumably hatched from eggs laid at the beginning of the spawning period in October, the previous year. A smaller sieve size

showed that the majority of the first settled individuals had not achieved 1mm length in Apr., five months after nidus formation, suggesting a long pre-settlement stage and initial slow growth. Farnie (1924) found that the embryo required at least twenty one days before it became free swimming. In the present study larvae hatched after 8 days but were still free swimming 21 days after egg laying. These observations suggest a long larval life although this has not been examined under field conditions.

During the five months (Apr. to Aug.) following recovery of the first individuals of the 0-group, numbers would be expected to increase according to the nidus formation pattern of the previous summer months. This was not observed but numbers present dropped after Apr. and then remained relatively constant during the winter. A large increase was observed in Sep. producing maximum numbers in Sep., Oct., and Nov. This is not easily explained but must be the result of settlement rates, migration, and changes in rate of size increase of small individuals after a winter period of slow growth. A number of studies of molluscs with planktotrophic larvae have shown that settlement may be delayed until a suitable environment is found (Newell 1970). Winter conditions probably also delay development, and Kiseleva (1979) has suggested that for some bivalves the growth rate of newly settled individuals may be depressed if population density and the ratio of size groups are optimal.

The constant mode of shell length from Mar. to Jul, implied that growth was balanced by loss of larger individuals, and immigration or development of smaller individuals. From Aug. to Oct. the total numbers increased to a maximum, and then decreased to a minimum from Nov. to Jan. The constant weight-

length mode, and proportional increase in weight of sizes larger than the mode, from Sep. to Dec. 1976 showed that this was the period of fastest growth, as well as of maximum increase in density. The increase in 4mm animals in spring agreed with Briggs (1972) who observed the first appearance of juveniles, 4mm long, in the rush beds in Sep. During Nov. to Jan. the density increased but the size mode increased, resulting from a decreased immigration or development of very small sizes, and large overall losses by mortality or emigration. The coefficients of rates of population change for the small individuals were of the same magnitide as those calculated for four mollusc populations in a river (Gillespie 1969).

There appears to be a loss at stations 1 and 2 of all animals above 8mm and of a large proportion of all small sizes throughout the year. Without replacement by settlement and development during the summer this resulted in a drop to a low level before the first settlement again. The possible causes of this loss were mortality from predation or a lethal factor such as toxic pollutants, or by emigration. Predation on *Amphibola* is thought to be limited to indiscriminate bottom feeding fish such as sand flounder, yellow-bellied flounder and yellow eyed mullet (Webb 1973). Birds were never observed feeding on *Amphibola*. Several Cominella glandiformis were often observed feeding on fresh dead adult *Amphibola*, and some natural mortality of large adults at stations 1 and 2 was observed in winter conditions. The absence of recognisable small dead shells suggests that if predation is the cause of the loss, then the predator crushes the shell. Mortality from chemicals associated with the outfalls does not explain the same losses of small sized

individuals at stations 3 and 4. The absence of other areas of extensive spatfall similar to this area on the western slopes, during the survey, Apr. to Jun. 1975, suggests that emigration from this area to the rest of the estuary may be significant. This also does not explain the loss of these sizes from stations 3 and 4. Some animals of the size interval 8 - 15mm were found around the estuary but there was an overall low density of this size. This may have been because of the timing of the survey which only recovered newly settled individuals or over 1-year olds, but the presence of small individuals at stations 1 and 2 during most months discounts the idea of an 0-group maintaining a discrete and exclusive size class through the year. If the 5 - 10mm size class is a dispersal phase then it is likely that would be concomitant with a high mortality from dispersal into unfavourable conditions. The coincidence of a high density and increased growth rate in an area of dense spatfall in Nov. could generate a level of competition for feeding space which triggers migration, producing a severe drop in numbers in Jan. This does not explain the same drop at stations 3 and 4 where spatfall was light, but the competition may have been provided by the high density of adults in this area.

Despite difference in latitude both Watters (1957) in Otago, and Briggs (1972) in Northland calculated the size after the first year's growth to be 8 - 13mm, and about 19.5 - 20.5mm after two years' growth. This was based on size frequency modes and the growth of marked individuals. In this study the numbers of 1mm individuals reached a maximum in Nov. when the first of the following seasons nidi were being formed which does not confirm a first year's growth to 8 - 13mm. This may be explained

by the sampling methods of Watters and Briggs which did not include sieve recovery of small sizes and both extrapolated back for sizes less than 8mm. Watters first size frequency mode of 8mm in Jun. 1957 was almost certainly a shoulder on a smaller size class mode. The present study showed that even a 1mm sieve produced a distorted size frequency mode in the first year. It is coincidental that the first appearance of 4mm individuals in Northland and a large increase in numbers of 4mm individuals in the Avon-Heathcote estuary should both be observed in Sep., even though the peak for nidus formation was two months earlier in Feb. in the more southern estuary. An exchange of genetic material along the coasts would be facilitated by the long larval life, and would explain the apparent lack of speciation throughout the species' geographic range. Because of the northerly current system along the east coast of the South Island, however, it is unlikely that the large influx of juveniles in Oct. and Nov. was comprised of immigrants from northern estuaries with later spawning periods.

It is interesting that the pattern of settlement and population changes during the year were the same at all stations, but settlement was consistently highest at station 2, slightly lower at station 1, and much lower at stations 3 and 4. This simultaneous increase at all stations contrasts with Briggs (1972) who showed an increase of small sizes on the sandflats which corresponded to a decrease on the mudflats during winter storms in July. Watters (1964) confirmed Farnie's (1924) suggestion that the veligers required solid substrate for settlement. This did not appear to be a requirement or explain the dense settlement on the western slopes of the Avon-Heathcote estuary.

It appears that a combination of factors, probably including silt-clay-organic content, and interstitial nutrients draining through the sediment from the sewage ponds, make the western slopes a preferred substrate for settlement. The animals, during subsequent early growth, are dispersed, perhaps by becoming buoyant, throughout the estuarine range. The absence of 8 - 20mm individuals, and the presence of only very large individuals at stations 1 and 2 was not explained.

A number of studies (e.g. Bruce 1953, Kilner 1969) have commented on an increase in density of adult *Amphibola* away from the sewage outfalls in the Avon-Heathcote estuary. In this study, station 1, closest to a main outfall, had a higher density of large *Amphibola* than station 2, which was in contrast to the density of newly settled individuals. Stations 3 and 4 had a very stable population in respect of density and size of larger individuals. There was no seasonal pattern and it appeared that growth was balanced by mortality or emigration of the largest individuals, and immigration of smaller individuals. This did not occur at specific times of the year and was not identified by shifts in the mode or range. A gradual increase in mean individual weight suggested a slowly aging population which may relate to changes in a physical factor such as sediment, which also caused an overall decrease in the number of small individuals settling in the area after 1974. Macpherson (1977) described net erosion occurring across this area, which may be gradually changing its suitability for some size classes of *Amphibola*.

2. Comparison of size frequencies at other areas of the estuary

Distinct differences in size frequency of *Amphibola* were found at different areas, and at stations 1 to 4 frequency structure was stable with a consistent annual cycle. This situation implies a predictable size-dependent response to physical or biotic factors. Behavioural selection involves an active or passive disperal process and an ability to maintain position within a selected habitat. It is possible that the burying response to the incoming tide minimises dislocation by water movement as adults would be vulnerable to turbulence at the sediment surface, because of shell shape. Adult *Amphibola* have a mobility range of about 2m per tidal period (Watters 1964, and observations in the present study) but this was determined from individuals which presumably were maintaining their position and not actively migrating. Annual migrations of fresh water pulmonate gastropods, induced by seasonal environmental changes, have been described in a number of studies (Cheatum . 1934). Even relatively sessile molluscs are able to move over the substratum after initial settlement to seek out a more suitable habitat (Paine 1971). Most studies of orientation by intertidal gastropods have sought a mechanism and cues which relate to coastal zonation according to tidal level (Gendron 1977), where physical factors vary along a single gradient normal to the shore. No studies have been found which describe selection mechanisms in an estuarine situation where gradients are more complex. In this study the estuarine survey showed no distinct zonation, but 1 - 10mm individuals were more common above M.W.L. and 10 - 20mm individuals were distributed in relation to the lower reaches of

the rivers. The highest densities of adults were found at M.W.L. but the broad distribution of all size classes shows that *Amphibola*, though having preferences, has a wide tolerance within its range. This makes it difficult to define factors significant to its distribution. Exposure, salinity, sediment particle size, organic content and pollutant toxicity are all likely to affect a preferred habitat. The high density of juveniles, and relative absence of adults at station 2, however, cannot be explained by any of these factors alone, as similar conditions in respect of each factor could be found at numerous other locations with different size frequencies.

The possibility of an effect of pollution at stations 1 and 2 was not clarified by detailed transects at stations 1 and 3. At station 1 a broader range of sizes occurred down the shore towards M.W.L., with an overall decrease in small sizes and an increase in fine sediment content. At station 3 a wide range of sizes occurred up the shore towards H.W.L., with an overall increase in small sizes, and a relatively small increase in silt-clay content, and still less than at stations 1 and 2. Water content did not seem to be a factor as surface water lay in low lying areas at all the stations (1 to 4).

The complexity of gradients of different factors makes it difficult to define a directional cue which would orient active migration. Thaids which responded to gradients of light and beach elevation to establish and maintain shore-level size gradients, showed a lack of significant size gradients on beaches of irregular topography (Bertness 1977). Small *Amphibola* showed a marked preference for sediment from station 1 over that from station 3, in the laboratory. The significant factor may be

particle size, organic carbon, or an unknown factor such as water chemistry. The feeding mechanism appeared to be indiscriminate with respect to sediment, and the only way in which *Amphibola* can choose its food is to choose its locality. A selection mechanism may be confused by the complex topography and benthic environment of the Avon-Heathcote estuary.

A relationship between biomass and organic matter is affected by the metabolic requirements of the biomass unit, depending on whether it comprises many rapidly respiring young individuals or a few old large individuals. The area carrying the largest biomass was not the area with the highest organic content. Metabolic rate (VO_2) has been related to body weight for *Amphibola* (Shumway, S.E. pers. comm.)

$$\log VO_2 = \log aW^b$$

where a = 0.108 - 0.158

b (slope) = 0.43 - 0.46

This relationship can be used to adjust the biomass loading according to the size of the individuals, and shows that, for example, 100 individuals each of 0.01g have a similar O_2 consumption as 10 individuals each of 2g, although the 10 individuals comprise twenty times the biomass of the smaller animals. This comparison would be further emphasised under natural conditions during Sep. to Dec. when growth of the small individuals was at a maximum. These considerations demonstrate the difficulty of relating biomass to environmental factors for groupings with widely differing size frequencies and growth rates, within a complex environment.

3. Shell sculpture

Shell sculpture in some gastropods has been shown to be correlated with particular factors such as wave action and shore profile (Struhsaker 1968, Wigham 1975). A microscopic examination of the structure of the sculpture (Chapter 6) showed that degree of sculpture was related to shell deposition rather than being the inverse result of subsequent erosion. Heavy sculpture appeared to be laid down by an increase in mantle activity. It was expected that smooth shells might predominate where stress from exposure, food supply, or pollution reduced mantle activity. There was no distinct spatial distribution of sculptural values which could be related to these factors, and the only trend was a slight increase in shell sculpture at stations 3 and 4 during spring. This may be the result of increased growth and mantle activity at that time.

CHAPTER V

A HISTOLOGICAL STUDY OF THE REPRODUCTIVE CYCLE

I. INTRODUCTION

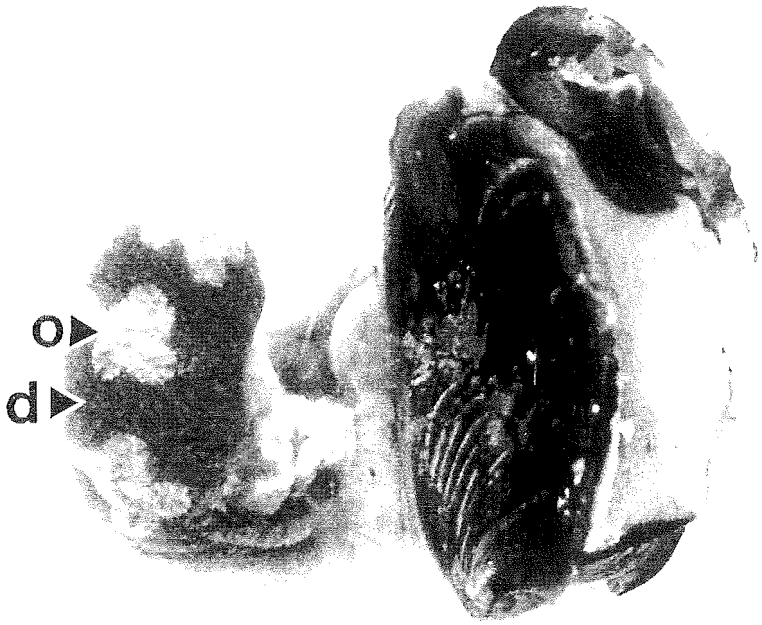
Amphibola crenata is hermaphrodite, with the ovotestis occupying a greater or lesser part of the visceral spire, depending on the size of the individual and the season. The ovotestis consists of clusters of light coloured finger-shaped follicles (acini) darkened at the tips, and alternating with bands of dark grey-coloured digestive gland (or liver) (Fig. 5.1.). The reproductive anatomy and early development of A. *crenata* was studied by Farnie (1919, 1924) who also reviewed and corrected discrepancies amongst earlier descriptions of the reproductive system. Farnie, however, commented only briefly on the seasonal development of reproductive products. She observed that spermatozoa were fully developed in Nov., when ova were still small, and that spermatozoa and ova developed in different regions of the same follicles. There have been no subsequent studies on the reproductive biology of A.*crenata*.

In the present study, the seasonal development of ova and spermatozoa in A. *crenata* was determined in individuals collected

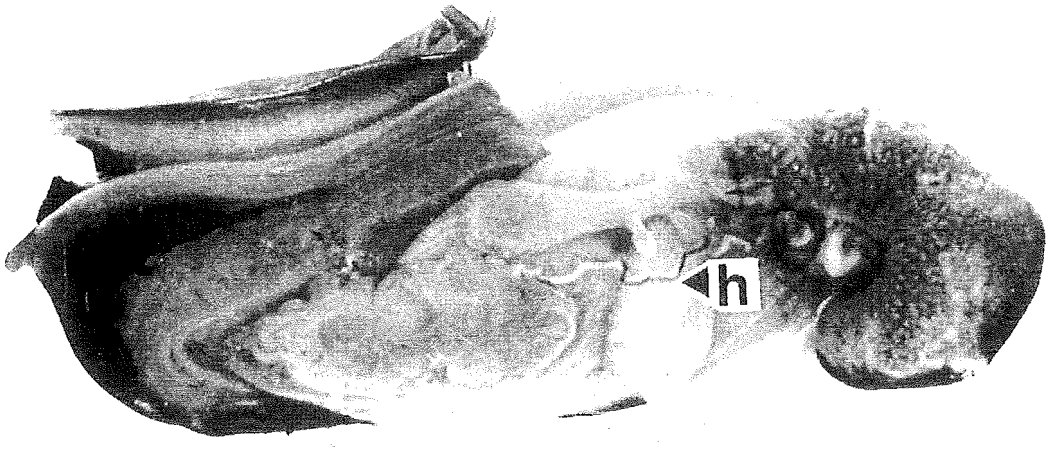
Fig.5.1.(facing) Soft body of *Amphibola crenata* after removal of the shell,

Showing
(a) the natural spiral configuration of the body, and
(b) the outstretched body, ventral side uppermost.

Bands of ovotestis and digestive gland alternate with each other along the visceral coil. The ventral hermaphrodite duct can be seen passing anteriorly from the ovotestis.
× 7. o - ovotestis, d - digestive gland,
h - hermaphrodite duct.



a



b

from both eastern (stations 1 and 2) and western (stations 3 and 4) slopes of the Avon-Heathcote Estuary. Results from these five areas could then be compared to investigate possible effects of proximity to sewage effluent on gamete development. Heavy metals and organochlorines have been shown to affect enzyme systems (Hewitt and Nicholas 1963, Risebrough et al 1968), and reproduction is likely to be affected by alterations in enzyme activity because it is closely controlled by enzyme pathways (Waldichuk 1974).

II. METHODS

At least five *A. crenata* of shell length greater than 12mm were collected at each of stations 1 to 4 approximately monthly from Feb. 1975 to Dec. 1976. Animals were kept at 15°C for 24h to eliminate sediment from their guts. Shell length was measured to 0.1mm with sliding calipers, and the shell was then broken with a hammer and the soft body carefully removed. After blotting the tissue dry, soft wet weight was measured to 0.01g on an electric balance. The soft body was fixed and preserved in 10% formalin in seawater for up to 18 months.

Prior to sectioning, the visceral coil was removed and transferred to 70% ethanol overnight, dehydrated by three changes of absolute ethanol over 24h, and immersed in cedar oil for clearing. Prior to embedding, cedar oil was removed from the tissue with xylene. After embedding, the wax block was trimmed and oriented in a rotary microtome (American Optical Company) to obtain 7mm thick transverse sections of the visceral coil. Twenty-four representative sections from each animal were mounted on a single slide, six serial sections being taken across four randomly selected areas along the coil. The pieces of ribbon were floated out with 2%

albumen solution and flattened over a flame. After removing wax the sections were stained in Lillie-Mayer Haemalum and eosin.

The sections of each individual were examined and the average percentage of visceral coil cross-section occupied by gonad was estimated. When present, the percentage of follicle cross-section occupied by spermatozoa was recorded. Five follicles and five oocytes were selected randomly and measured at 400 x mag. This provided a minimum of 25 regular monthly measurements for each dimension at each station. Only oocytes which showed a nucleus were measured to avoid underestimation of oocyte diameter from acentric sections.

Gonad tissues were carefully examined for cellular abnormalities or neoplasms of the type observed in ovaries of *Mercenaria mercenaria* (Yevich and Barry 1969). The extent of parasitic infection of the visceral coil was recorded for each animal.

III. RESULTS

(1) General Description of Seasonal Cycle

Ovotestis and digestive gland occurred in alternating bands along the visceral coil to the tip of the spire. Ovotestis tissue comprised ellipsoid (in longitudinal section), circular (in cross section) follicles connecting with ducts radiating from the ventral hermaphrodite duct and extending out to the dorsal perimeter of the visceral coil (Figs 5.2., 5.3.). Sperm and ova were never found in individuals with a shell length less than 20mm but small follicles and spermatogonia or primary sperm cells were present in all individuals down to 12mm, the smallest size examined (Figs 5.4., 5.5.). Oogonia were not identified in animals less than 20mm long.

In mature adults spermatogonia were present throughout the year. Spermatocytes developed through the winter, showing as dark stained clusters arranged around 'basal' or nurse cells, and extending into the follicle from the germinal epithelium (Figs 5.6., 5.7., 5.8.). By Sept. these clusters occupied most of the follicle lumen and spermatids appeared (Fig. 5.10.). This was also the site of oogenesis. When oocytes were present spermatids developed along the epithelium separating oocytes from the follicle lumen. This gave rise to a concentric arrangement around the duct with developing oocytes along the perimeter, spermatogenesis sites along the outside edge of the follicle lumina, and parallel 'tails' of cytoplasm streaming out normal to the follicle wall into the lumina (Figs 5.12., 5..13.).

Mature sperm detached from the epithelium in clumps and appeared in the follicular lumen with parallel cytoplasmic 'tails' attached to dark staining bands of follicular cells (Fig. 5.14). After detachment sperm heads were distinguishable at the ends of the sperm distal to the follicle wall site of previous attachment. Sperm then moved into the ciliated ducts, discarding the follicular cells and appearing as a tangled mass in the common hermaphrodite duct (Fig. 5.14.). Follicular lumina and the hermaphrodite duct were packed with spermatozoa by Nov. (Figs 5.11., 5.13.). This duct was distended with sperm throughout the breeding season from Oct., Nov. (Fig. 5.14.) when the first nidi were observed on the sand.

Following the summer cycle of gametogenesis and gamete extrusion old yolky material and sperm debris were phagocytosed, and oocytes replaced by a proliferation of yellow staining bodies of globules (Figs 5.15., 5.16.). It has been suggested that these

bodies are either yolk granules or gland cell goblet contents (Morton, J.E., pers. comm.). They were present during the breeding season as small isolated yellow regular-shaped spheres always associated with oocytes. During the winter when follicles contracted, these bodies completely filled the follicles or aggregated around the distal wall of the follicle where oogenesis had occurred the previous season (Figs 5.7., 5.15., 5.16.). They were also present during the non-breeding period around the wall of the otherwise empty hermaphrodite duct (Fig. 5.17.).

Occasional small oocytes were observed during winter, some indicating a re-initiation of oocyte production following the breeding season (Fig. 5.6.), or early initiation of production after Jun. Oocyte development mainly took place after Nov. and most were not fully mature until the end of Dec., two months after sperm were produced into the hermaphrodite duct and the beginning of nidus production. Fully developed oocytes were rarely seen in the follicle lumen and were never observed in the hermaphrodite duct. The probability of observing free oocytes is low because of their comparatively low frequency and short residence time in the ducts.

Large highly vacuolated interfollicular cells were observed throughout the year but predominated in sub-adults during the non-breeding period to occupy the space around the small, or contracted, follicles.

Sperm were observed in the bursa copulatrix and vas deferens in Dec. (Fig. 5.20.) and the histological appearance of two glandular portions of the genital duct is shown in Fig. 5.21. These glands are comprised of a combination of columnar glandular and ciliated cells which is typical of genital gland tissue.

Photographs on facing page

Fig.5.2

Fig.5.3

Fig.5.4

Fig.5.5

Fig.5.2. Tip of visceral coil showing ducts lined with ovotestis follicles radiating from the ventral hermaphrodite duct, and alternating with segments of digestive gland follicles. Shell length - 23.4mm, collection date - Apr.1975, x 45

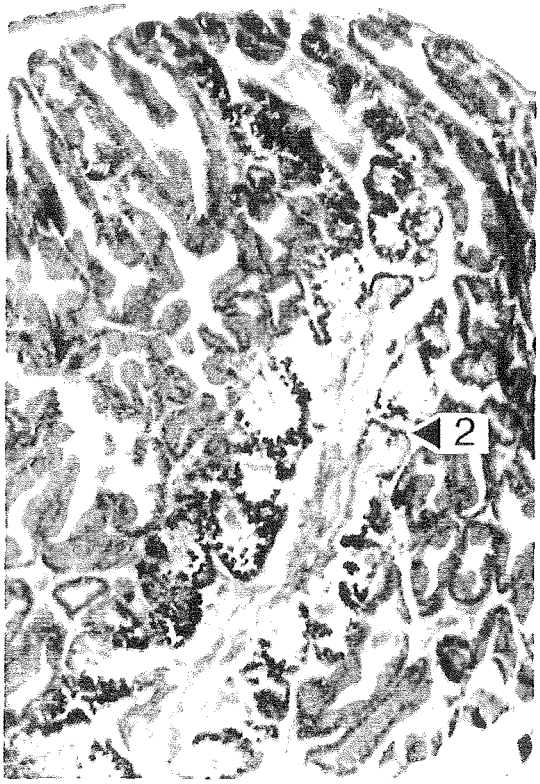
Fig.5.3. Ovotestis follicles opening into a duct connecting with the hermaphrodite duct.
Shell length - 21.4mm, collection date - Jan.1976, x 90

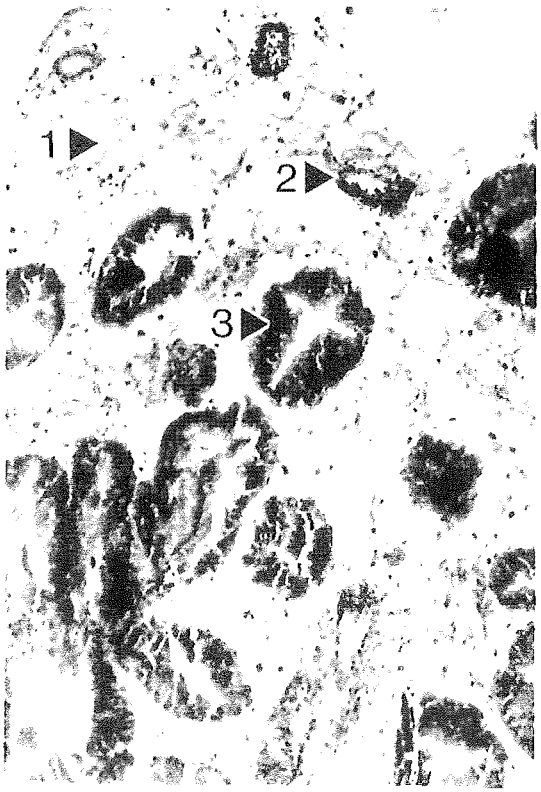
Fig.5.4 Small undeveloped ovotestis follicles surrounded by interfollicular cells. Dark stained spermatogonia are present around the germinal epithelium.
Shell length - 14.4mm, collection date - Aug.1976, x 230

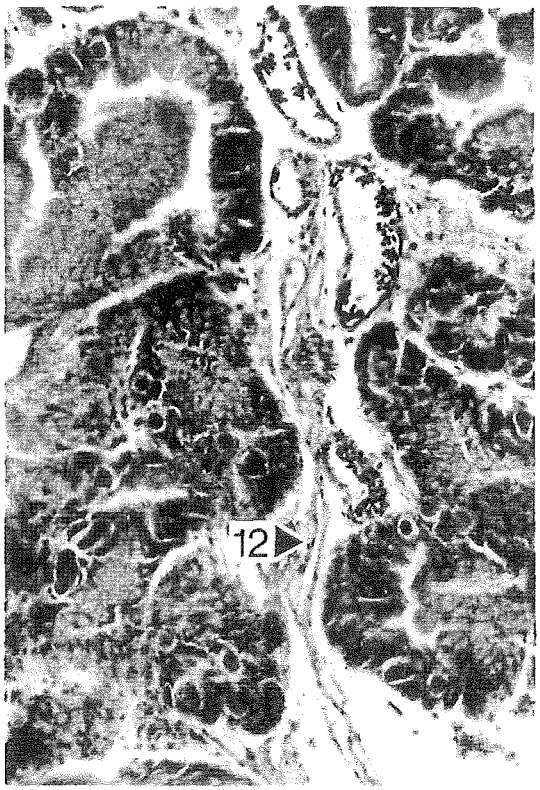
Fig.5.4. Ovotestis follicles along a duct in a sub-adult. Clusters of spermatocytes did not develop into spermatids.
Shell length - 16.1mm, collection date - Jan.1976, x 345

1. interfollicular cells
2. ovotestis follicles (acini)
3. digestive gland follicles
12. duct connecting ovotestis follicles with hermaphrodite duct


3
2


2


1
2
3


12

Photographs on facing page

Fig.5.6

Fig.5.7

Fig.5.8

Fig.5.9

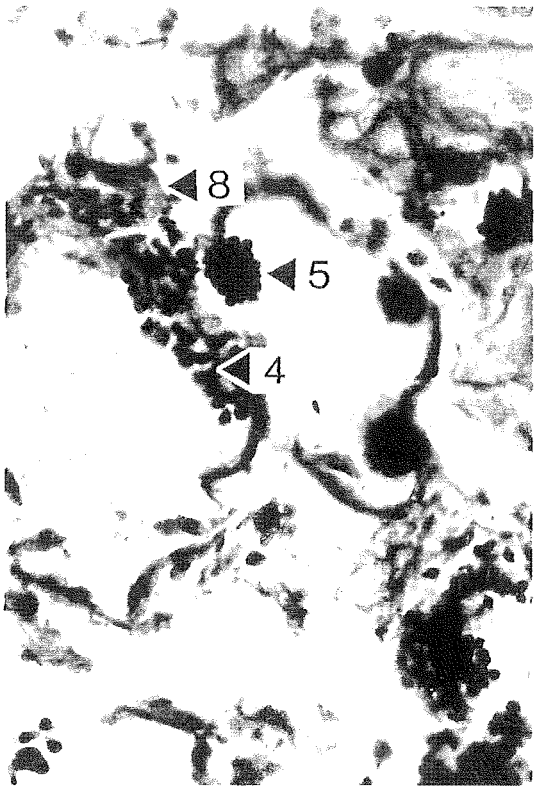
Fig.5.6. Spermatogonia, and spermatocytes developing around basal cells. A small immature oocyte suggests re-initiation of oogenesis following the preceding breeding season.
Shell length - 19.1mm, collection date May 1976, x 700

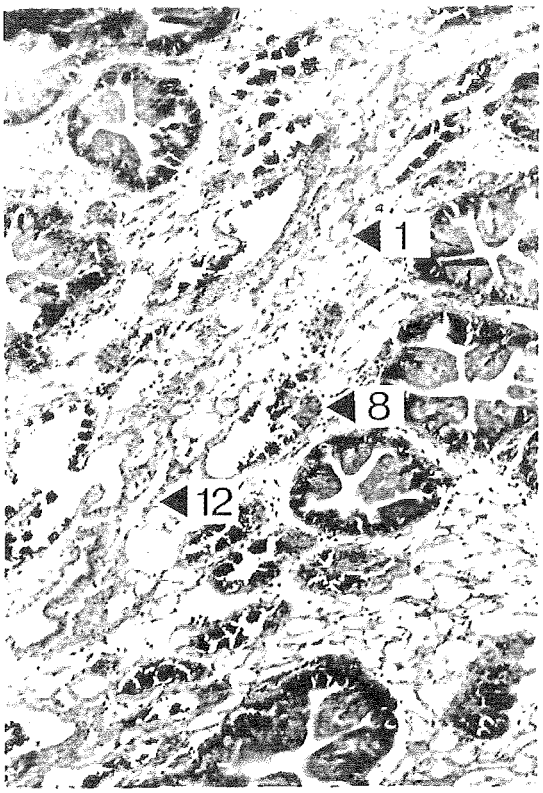
Fig.5.7. Ovotestis follicles with clusters of spermatocytes, and oogonia surrounded by yellow staining bodies. Oogonia occur at the distal ends of the follicles while the epithelial cells of the follicle wall proximal to the duct, are ciliated.
Shell length - 27.6mm, collection date - Jul.1975, x 115

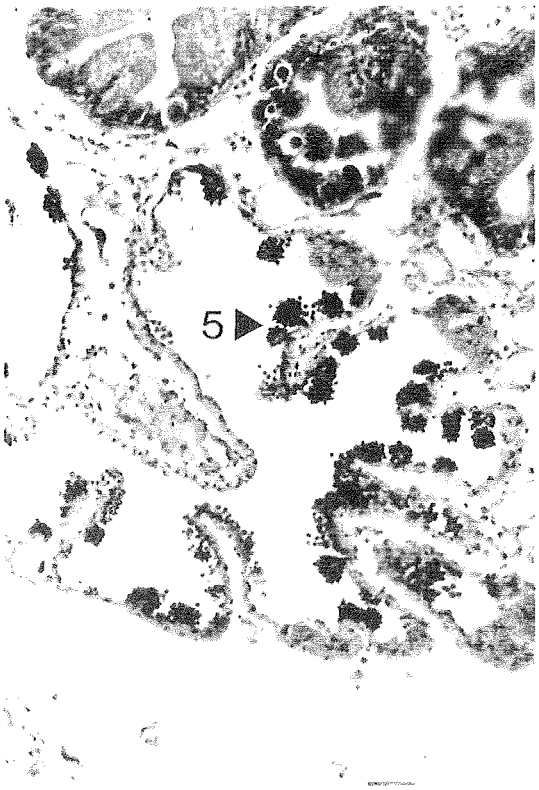
Fig.5.8. Detail of an ovotestis follicle as shown in Fig.5.7.
Shell length - 27.6mm, collection date - Jul.1975, x 230

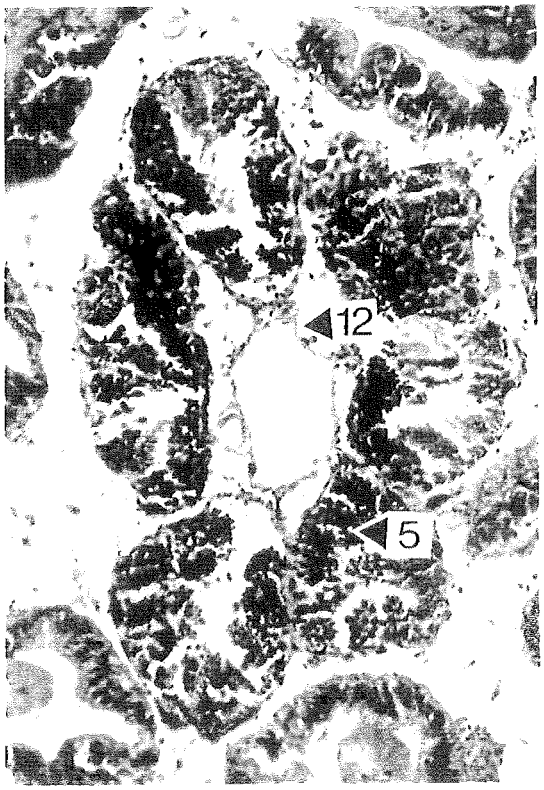
Fig.5.9. Ovotestis follicles around a duct with fully developed spermatocytes and spermatids extending into the follicle lumen. Spermatozoa are beginning to detach from the germinal epithelium distal to the duct.
Shell length - 20.3mm, collection date - Sep.1975, x 230

1. interfollicular cells
4. spermatogonia
5. spermatocytes
8. developing oocyte
12. duct connecting ovotestis follicles with hermaphrodite duct


8
5
4


1
8
12


5


12
5

Photographs on facing page

Fig.5.10

Fig.5.11

Fig.5.12

Fig.5.13

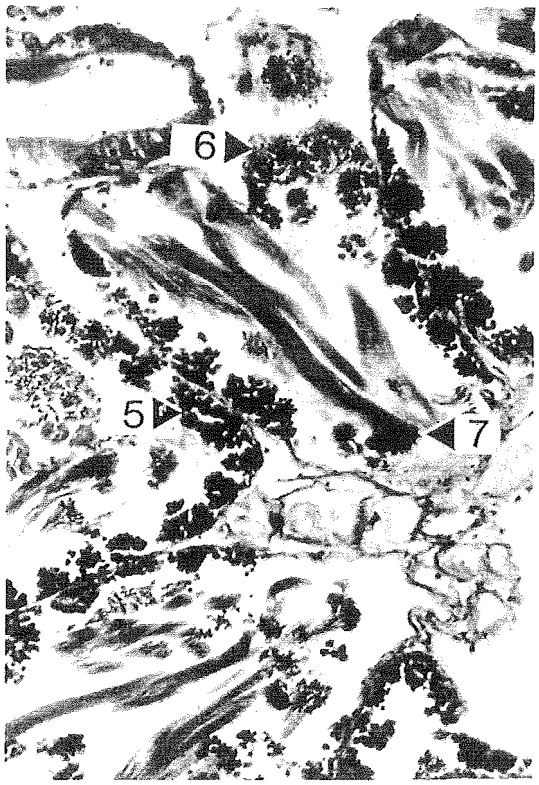
Fig.5.10. Mature sperms detaching in clumps from the epithelium. Dark bands of cells indicate where sperms have detached from the wall while sperm heads are distal to the detachment site.
Shell length - 20.9mm, collection date - Nov.1976, x 230

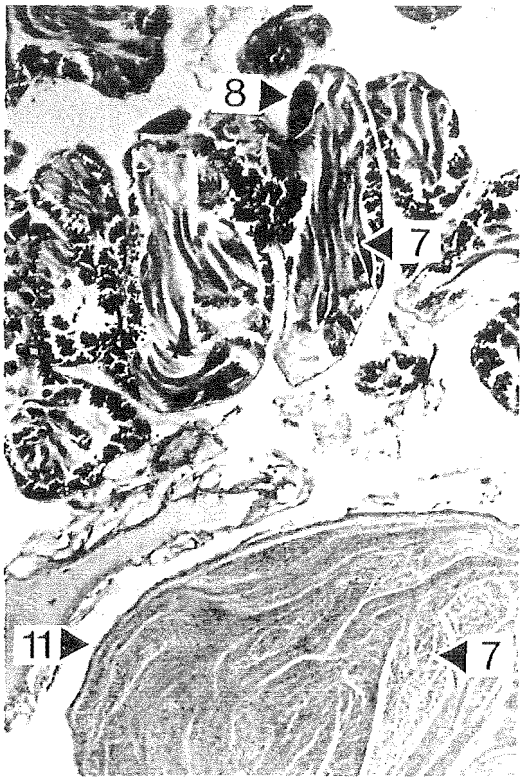
Fig.5.11. Oocytes developed during Oct., Nov., after hermaphrodite has filled with sperm.
Shell length - 24.9mm, collection date - Nov.1976. x 115

Fig.5.12. Concentric arrangement of oocytes on the perimeter of the follicular epithelium with sperms detaching from the inner wall. Cytoplasmic 'tails' of spermatids stream into the follicle lumen towards the central duct.
Shell length - 26.9mm, collection date - Feb.1975, x 115

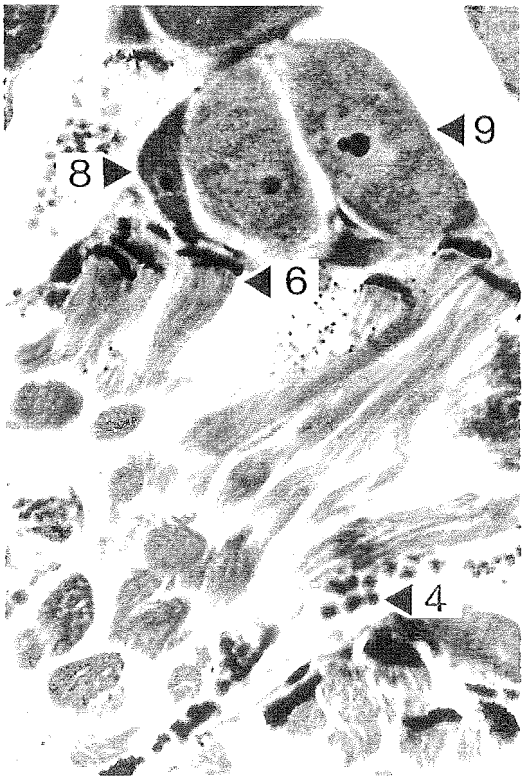
Fig.5.13. Spermatids attached to the epithelium surrounding a group of oocytes on the perimeter of the follicle. Nuclei show clearly in the oocytes and a darker stained immature oocyte lies to the left of two mature oocytes.
Shell length - 22.8mm, collection date - Feb.1975, x 460

4. spermatogonia
5. spermatocytes
6. spermatids
7. spermatozoa
8. developing oocyte
9. mature oocyte
11. wall of hermaphrodite duct
12. duct connecting ovotestis follicles with hermaphrodite duct.


6
5
7


8
7
11
7


6
7
12
9


9
8
6
4

Photographs on facing page

Fig.5.14

Fig.5.15

Fig.5.16

Fig.5.17

Fig.5.14. Spermatozoa packed the tightly convoluted hermaphrodite duct throughout the breeding season.
Shell length - 23.2mm, collection date - Jan.1976, x 46

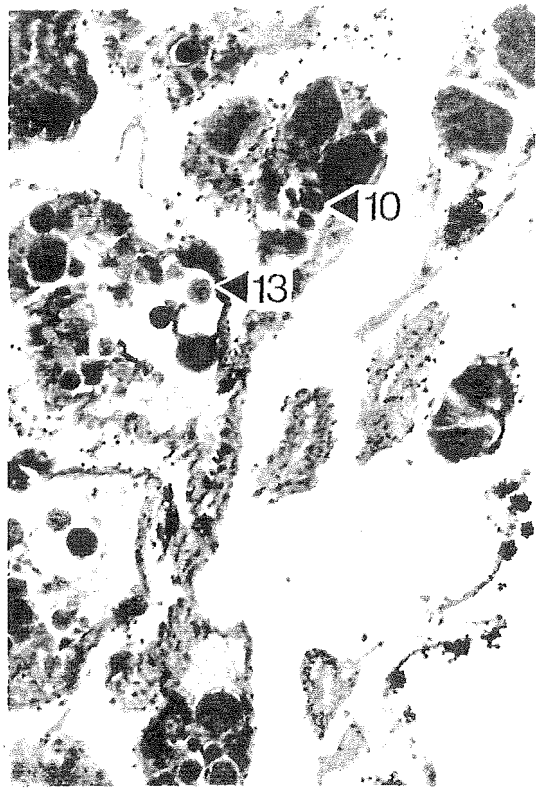
Fig.5.15. Oocytes disintegrated and were phagocytosed following the breeding season, with a subsequent increase in yellow bodies and contraction of the ovotestis follicles.
Shell length - 24.4mm, collection date - Mar.1976, x 230

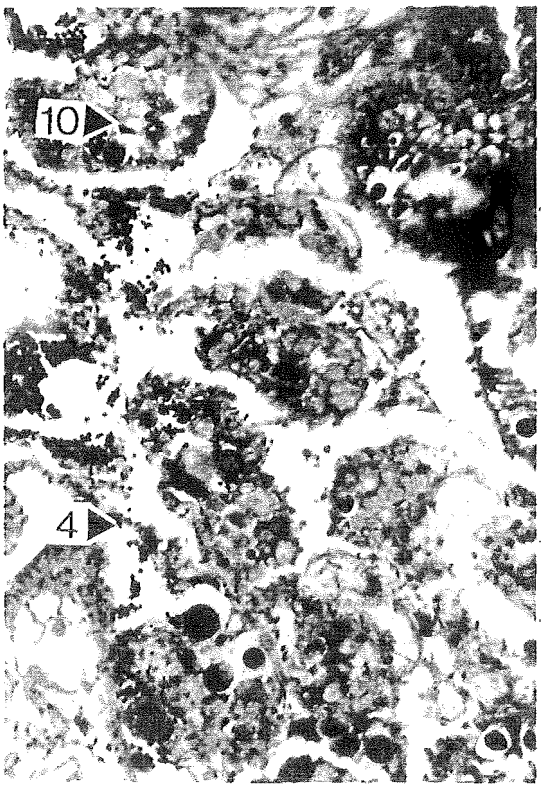
Fig.5.16. Yellow bodies packed the contracted follicles during the winter months. Dark staining spermatogonia can be seen around the follicular epithelium. Sperm debris and occasional oogonia were also seen during this period.
Shell length - 25.6mm, collection date - Jan.1976, x 46

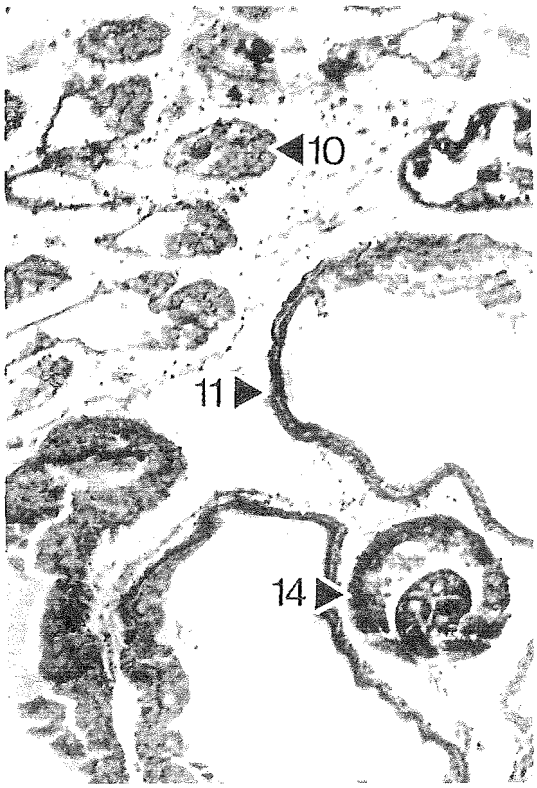
Fig.5.17. During winter sperm debris remained in the hermaphrodite duct lumen, and yellow staining bodies aggregated around the inside of the ciliated walls of the duct. Yellow bodies can be seen in both the follicles and the hermaphrodite duct. An encysted miracidium is located amongst the convolutions of the hermaphrodite duct.
Shell length - 26.6mm, collection date - Jun.1976, x 115

2. ovotestis follicles
4. spermatogonia
7. spermatozoa
10. yellow bodies
11. wall of hermaphrodite duct
13. site of phagocytosis of old yolk material
14. encysted parasite


2
7


10
13


10
4


10
11
14

Photographs on facing page

Fig.5.18

Fig.5.19

Fig.5.20

Fig.5.21

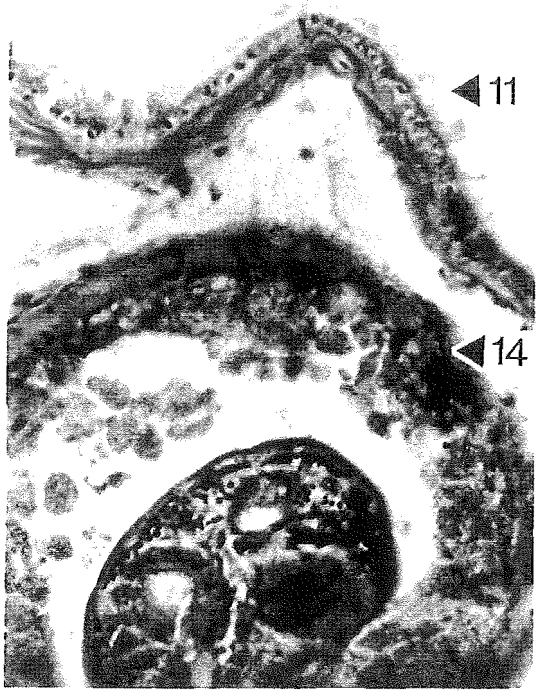
Fig.5.18. Wall of the hermaphrodite duct during winter, showing a thin layer of muscle cells around a ciliated epithelium. The lower half of the section shows detail of the encysted parasite stage in Fig.5.17. Shell length - 26.6mm, collection date - Jun.1976, x 460

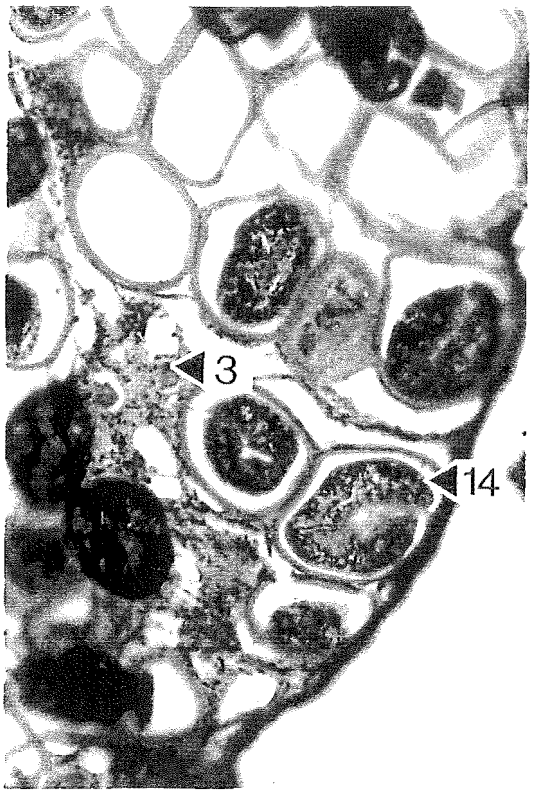
Fig.5.19. Chronic infection of the visceral coil by trematode sporocysts and rediae. Digestive gland follicular material can be seen amongst the cysts. Shell length - 18.1mm, collection date - May 1976, x 230

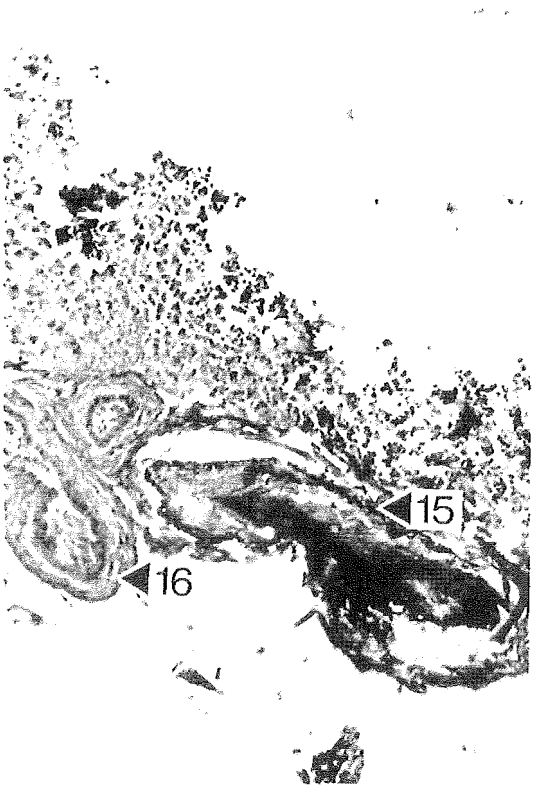
Fig.5.20. Bursa copulatrix filled with sperms which are oriented with the heads held on the wall of the sac by epithelial cells, perhaps preliminary to phagocytosis. Cross-sections of coils of the vas deferens are shown, as it proceeds internally to the base of the penis. Shell length - 22.2mm, collection date - Dec.1976, x 115

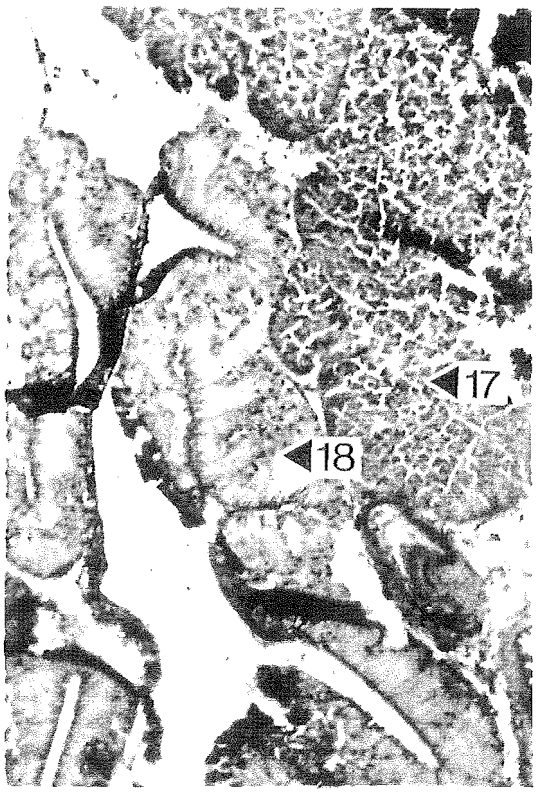
Fig.5.21. Mucous gland of the glandular genital tract alongside the albumen gland. Shell length - 22.2mm, collection date - Dec.1976, x 115

3. digestive gland follicles
11. wall of hermaphrodite duct
14. encysted parasite
15. bursa copulatrix
16. vas deferens
17. albumen gland
18. mucous gland


11
14


3
14


15
16


17
18

(2) Gonad Development and Follicle Size

The percentage of visceral coil of adults (greater than 20mm shell length) occupied by gonad dropped abruptly from 40% to 60% in Feb. to about 20% in Apr. in both 1975 and 1976 (Fig. 5.22). In 1975 gonad content was lowest, with percentage gonad higher at stations 1 and 2, but during gonad expansion in spring the situation was reversed. Follicle size did not show a seasonal pattern in individuals less than 20mm long, and remained at about 0.08mm.

Mean follicle diameter (Fig. 5.23.) in adults greater than 20mm long decreased from Feb. (mean, about 0.22mm) to a minimum in Jul. for 1975 of about 0.10mm. During Sep. follicle diameter began to increase at a similar rate and magnitude as gonad percentage (Fig. 5.22.). In animals less than 20mm long, gonad remained below 20% throughout the year and did not show an increase related to breeding season. Difference in follicle diameter between months, and within months between stations, was tested for significance by analysis of variance (Table V.1.). The raw data required a log transformation to fit a normal distribution. The only significant difference was between months, and Duncan's new multiple range test was used to separate months at the 0.01 level (Table V.II)

The relationship between follicle diameter and shell length at stations 1 and 2 was compared with that at stations 3 and 4. This was done by comparing the correlation coefficients, and regression lines for the data from each area. These statistics represent means derived from seasonal changes in follicle size which tend to obscure the relationship with shell length. A positive correlation was found between log shell length and log follicle size (Fig. 5.24b) and about 20% of the variation in follicle size

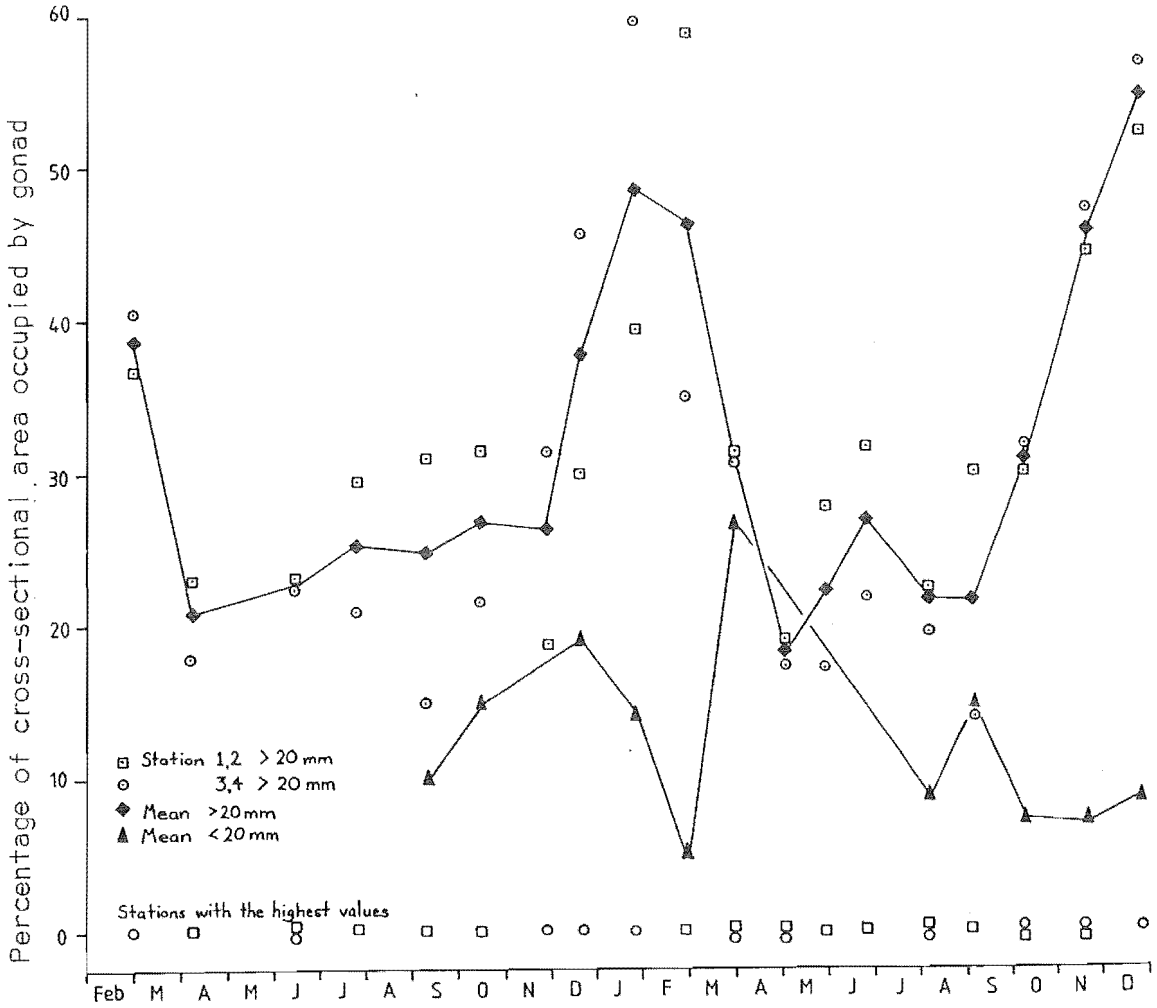


Fig.5.22. Percentage of visceral coil cross-sectional area occupied by gonad, in *Amphibola crenata* with shell length less than, and greater than 20mm. Values are means of at least 10 individuals combined from stations 1 and 2, as well as from stations 3 and 4, each month during 1975 and 1976.

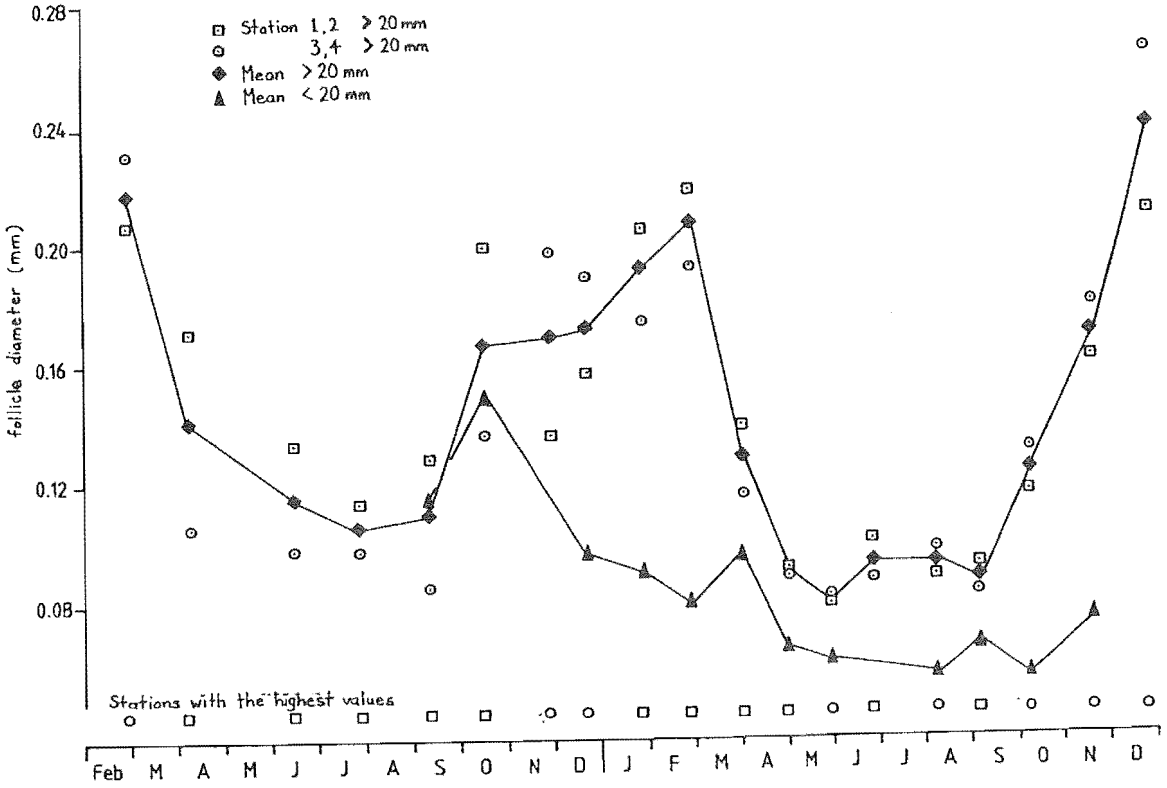


Fig.5.23. Ovotestis follicle diameter of *Amphibola crenata* with shell length less than, and greater than 20mm. Values for the larger size class are means of at least 5 follicles in each of 10 individuals combined from stations 1 and 2 as well as from stations 3 and 4, each month during 1975 and 1976.

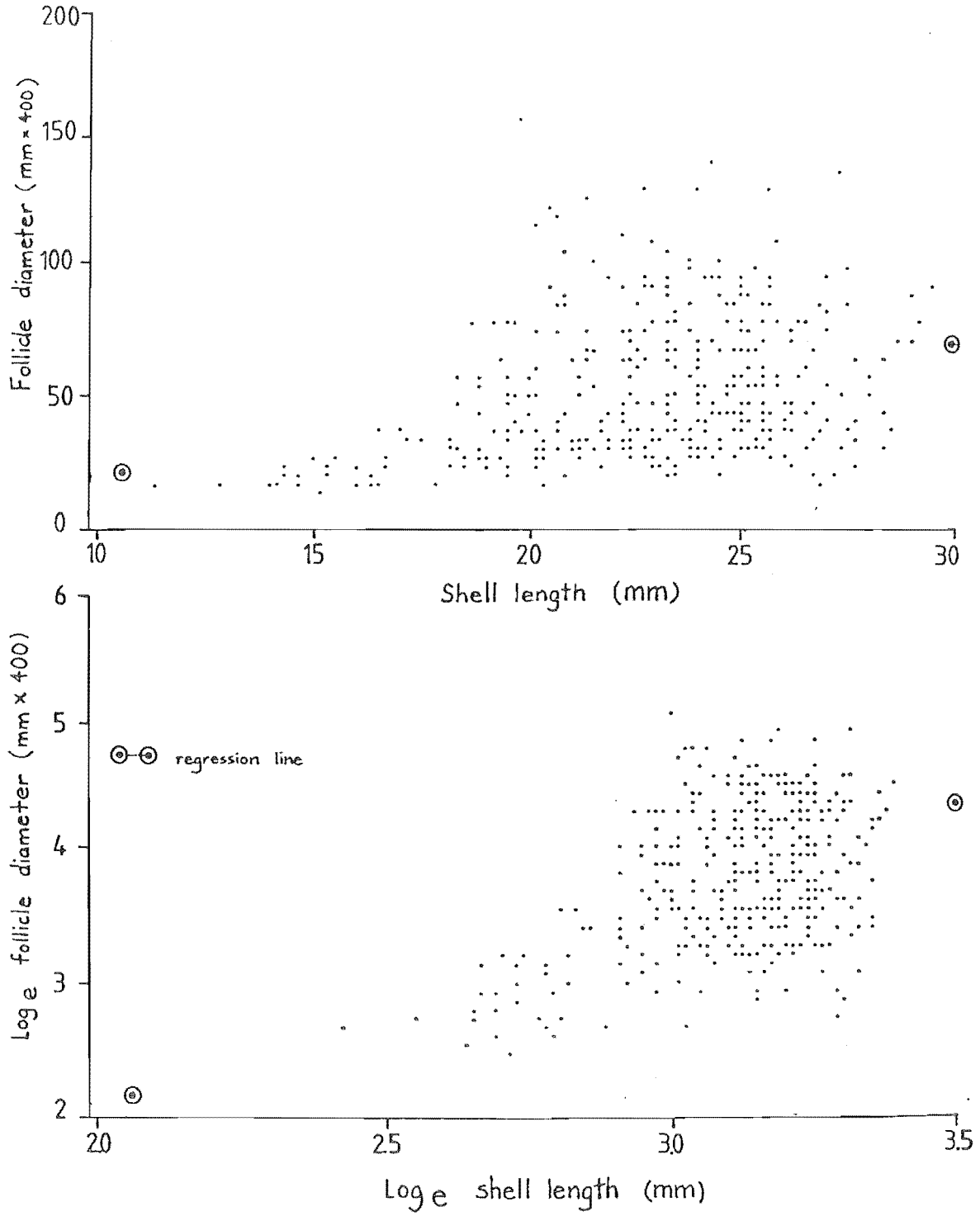


Fig.5.24. Relationship of follicle diameter to shell length for *Amphibola crenata* collected from stations 1 to 4, each month during 1975 and 1976. Computer plots of raw data and log transformed data are compared.

Table 5.I Ovotestis follicle diameter

Analysis of variance: months (H); stations 1,2(T_1), 3,4(T_2);
stations 1,2,3,4(S).
Normal distribution after log transformation.

	F	P	
M	20.6165	0.000	sig.
T	1.3347	0.367	n.s.
T against S	1.6145	0.201	n.s.
MT	1.6100	0.110	n.s.
MT against MS	1.5642	0.026	sig.

Table 5.II Ovotestis follicle diameter

Duncan's new multiple range test, p less than 0.01.
Months in each line are significantly different from
months in other lines.

		Jun.	Jul.	Sep.				Apr.	May	Jun.	Jul.	Aug.	Sep.
Apr.					Oct.	Nov.	Dec.	Jan.	Mar.						Nov.
Feb.'75									Feb.						Dec.'76

Table 5.III Shell length and ovotestis follicle diameter

Normal distribution after log transformation

Correlation coefficient

	R	P	
Station 1, 2(T_1)	0.3761	0.000	Probability that $R_1 = R_2$ is 0.1873
Station 3, 4(T_2)	0.4867	0.000	

Regression T_1 $\log_e Y = -0.5113 + 1.348 \log_e X$

T_2 $\log_e Y = -1.8428 + 1.8182 \log_e X$

where Y is follicle diameter (mm times 400) and
X is shell length(mm)

Difference between regression lines:

means	1.0829	0.2987	n.s.
slope	1.6317	0.2023	n.s.
error variance	1.1677	0.2861	n.s.

could be attributed to the effect of shell size (Table 5.III).There was no significant difference between regression lines or correlation coefficients of the two areas. The plot of raw data (Fig. 5.24a.) showed that follicle size began to increase in animals with a shell length above about 18mm.

(3) Oocyte Development

Numbers of oogonia and oocytes showed similar seasonal changes (Fig. 5.25a.). Both were absent during Jun. and Jul. 1975 but a few oogonia were found in Jun. and Jul. 1976. An increase in numbers of oogonia and oocytes began in Sep. and Oct. and reached a maximum in Feb. in both 1975 and 1976. Oocyte diameter (Fig. 5.25b.) showed similar seasonal patterns to oocyte numbers, although in 1976 diameter remained at a maximum from Jan. to Mar. while analysis of variance comparing differences in oocyte diameter between months, and within months between stations (Table 5.IV) showed that there were significant differences between months, and between stations 1, 2, 3 and 4 when they were tested separately against the combined stations in each area. There was no difference however between the combined data for these areas indicating that local variation was greater than differences which might be attributed to the effect of sewage. Duncan's test (Table 5.V) showed that oocyte size was significantly higher for three months of the breeding season. Oocyte production continued after the full cycle of gametogenesis and gamete discharge, and a few young oocytes were observed in April.

(4) Sperm Development

Spermatogonia were present throughout the year, decreasing in numbers after Jan. and then increasing again after May (Fig. 5.26a.).

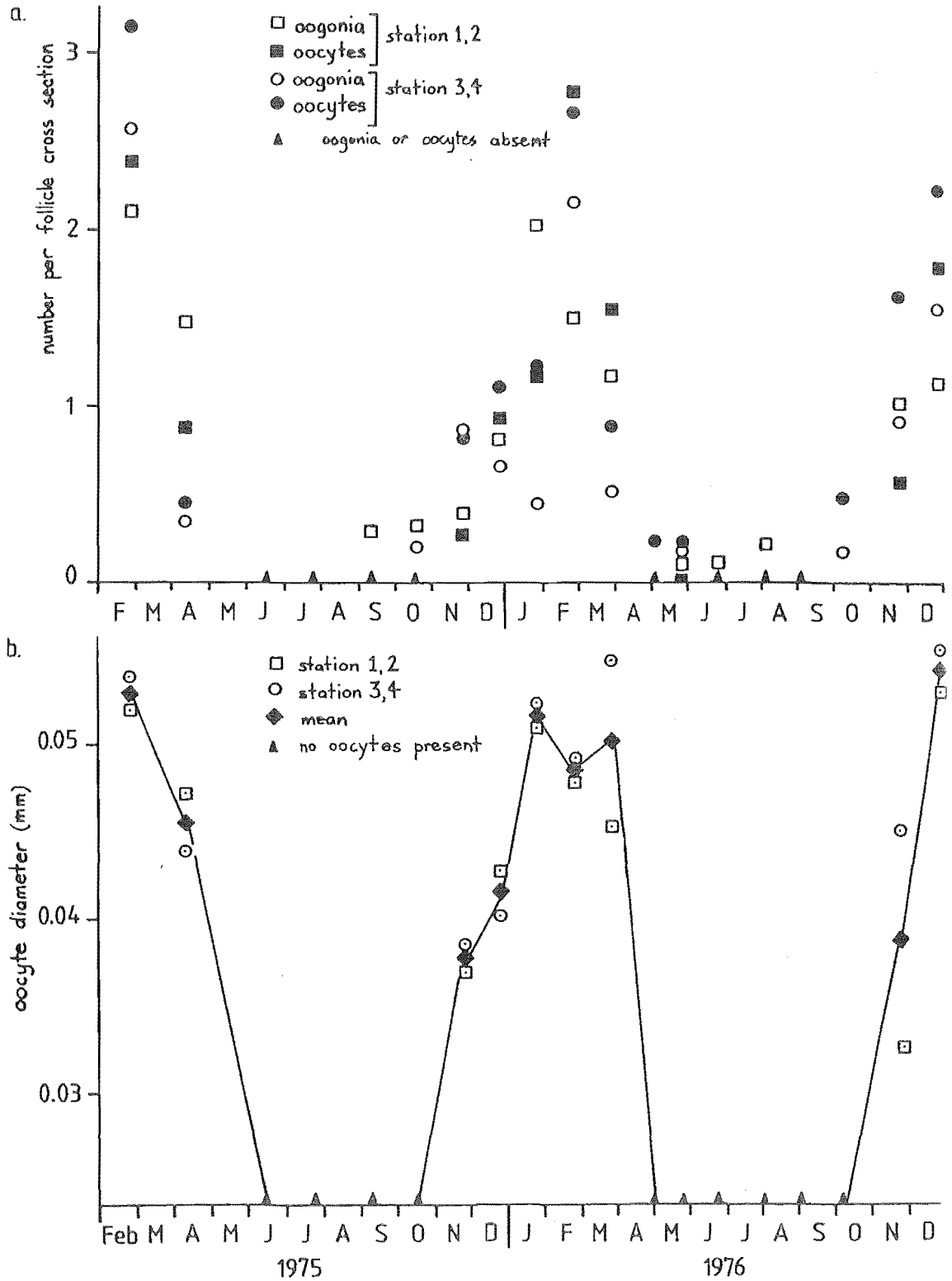


Fig.5.25.a. Numbers of oogonia and oocytes per follicle cross-section

5.25.b. Oocyte diameter

Values in figs. a. and b. are means of at least 5 random measurements in each of 10 individuals combined from stations 1 and 2, as well as from stations 3 and 4, each month during 1975 and 1976.

Table 5.IV Oocyte diameter

Analysis of variance: months (M); stations 1,2(T_1);3,4(T_2)
stations 1,2,3,4(S).
Raw data conformed to a normal distribution.

	F	P	
M	10.098	0.000	sig.
T	0.269	0.656	n.s.
T against S	15.606	0.000	sig.
MT	0.412	0.897	n.s.
MT against MS	3.705	0.000	sig.

T	1	2	
mean	17.806	18.864	(n.s.)

S	1	2	3	4	
mean	19.100	16.512	17.284	20.444	sig.

Table 5.V. Oocyte diameter

Duncan's new multiple range test, p less than 0.01.
Months in each line are significantly different from
months in other lines.

Feb.'75 Apr. Jan.Feb.Mar. Dec.'76
Nov.Dec. Nov.
Jun.Jul.Sep.Oct. Apr.May Jun.Jul.Aug.Sep.

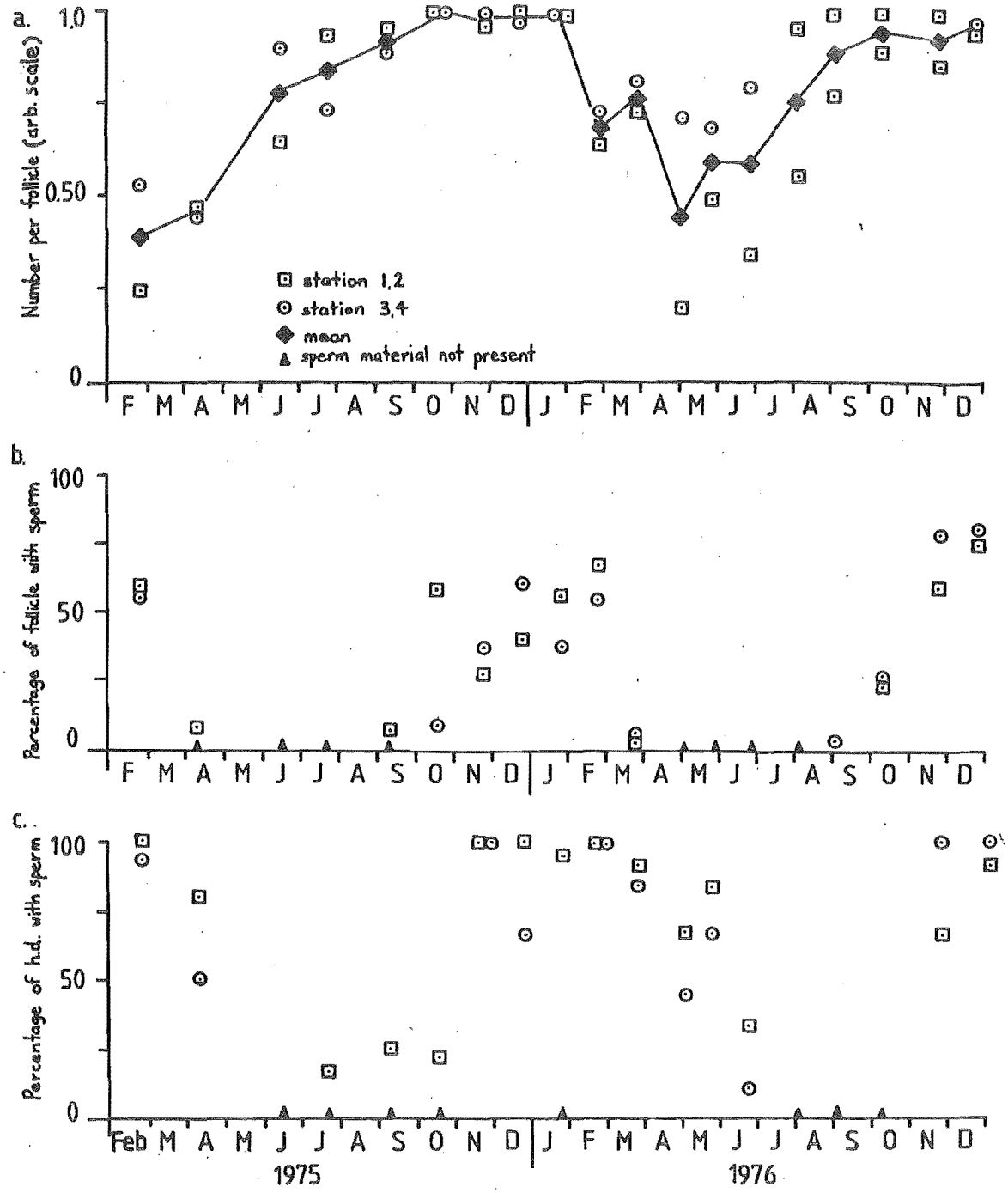


Fig.5.26.a. Numbers of spermatogonia and spermatocytes estimated on an arbitrary scale, 0 - 1.0, per follicle cross-section.

5.26.b. Percentage of follicle cross-sectional area occupied by spermatids and spermatozoa.

5.26.c. Percentage of hermaphrodite duct cross-sectional area occupied by spermatozoa. Sperm debris remained temporarily in the duct after the end of the breeding season.

Key in Fig.5.26.a. also refers to figs.b. and c.
Values are means of at least 5 random measurements in each of 10 individuals combined from stations 1 and 2, as well as from stations 3 and 4, each month during 1975 and 1976.

Primary and secondary spermatocytes developed during winter in both 1975 and 1976 and by Sep. the first spermatids were observed (Fig. 5.26b.). From Oct. to Feb. spermatocytes, spermatids and mature spermatozoa occurred together in the follicles. From Dec. to Feb. about 50% of the follicle was occupied by spermatozoa but this was all discharged by Apr. and no mature spermatozoa were observed in the follicles during the winter months. Sperm debris, however, persisted in the hermaphrodite duct until Jun. in 1976 (Fig. 5.26c.). There was no apparent significant difference in sperm development between the different stations.

(5) Parasitic Infection

Infection by an occasional isolated encysted miracidium (Fig. 5.17., 5.18.) embedded in the visceral coil between both liver follicles and ovotestis acini was observed at all stations during all months, and in 20% to 100% of the individuals of each sample.

The most acute infection occupying the whole visceral coil and comprising sporocysts and rediae (Fig. 5.19.), was observed mainly from Mar. to Jul., although isolated examples of chronic infection occurred during most months. Incidence of infection was similar at all stations. The trematode was not identified, but it is probably the same species as that observed by Farnie (1919) and Watters (1957).

IV DISCUSSION

Reproductive strategies in gastropods and the relationship between marine and freshwater species have been considered in a number of recent studies (e.g. Calow 1978, Russell-Hunter and

McMahon 1976). The relative advantages of protandrous succession (sperm first) and simultaneous or synchronous development of ova and spermatozoa, hermaphroditism, semelparity and iteroparity (one or several adult breeding seasons) and trends in egg size and number have been debated. The particular strategy evolved in a species has been evaluated according to the advantage that it confers on that species in relation to its environment. Most pulmonates are hermaphrodite but both simultaneous and protandrous conditions occur (Morton 1955, Russell-Hunter and McMahon 1976). Gastropods colonising freshwaters have tended to semelparity with a concomitant increase in egg size and decrease in egg number. Those with a short life span generally expend more effort per reproductive season, although one exception which has been studied is the salt marsh pulmonate, *Melampus bidentatus*. Calow (1978) has compared reproductive effort using an index (Indirect Effort Index, IEI) which compares total volume of ova released in a breeding season with volume of the adult shell. Values have been calculated for *A crenata*, based on an annual egg production of 100,000. The number of eggs laid in a season was derived from Briggs' (1972) estimate of 7,500 eggs per nidus, and the observation in the present study that one nidus is laid each 5 days, giving a total of 12 to 15 nidi per individual each season (Chapter 4). This is greater than Briggs' estimate of 5 to 6 nidi per individual each season. Estimated egg production is less than that calculated for some marine species, however, with very small eggs and planktotrophic larvae, e.g. *Mytilus edulis* 10^7 eggs (Fretter and Graham 1964). The value of IEI obtained for *A. crenata* (0,0128) is considerably lower than that calculated by Calow (1978) for several species with iteroparous populations (Table 5, VI), which

probably points to an exceedingly long breeding life in A. *crenata* and a long larval stage which allows each ovum to carry relatively low food reserves.

Table 5.VI. Average indirect indices of reproductive effort for different life cycle patterns (taken from Calow (1978), except for A. *crenata*).

	Average IEI	Egg size (mm)	Egg number
Semelparous species	2.06	0.7 to 1.3	34 to 1000
Iteroparous species	0.28	0.1 to 1.0	50 to 1000
Melampus bidentatus	0.89		33,000
Amphibola crenata	0.0128	0.08	100,000

To ensure survival in an estuarine environment a species can either lay a few eggs, each encased for development in a protected capsule, or partition the same energy into a large number of small eggs with planktotrophic larvae. In A. *crenata* the high loss of these larvae into unfavourable conditions in freshwater or out to sea is compensated by maximum colonisation of the intertidal estuary environment, and the facilitation of genetic exchange between estuaries. The long adult life span of A. *crenata* is characteristic of a species in which there is intense intraspecific competition for space and food (Pianka 1970). This is probably true for large areas of intertidal mud flats where A. *crenata* reaches high densities. Its occurence in high densities and its relatively high mobility renders hermaphroditism of small importance for facilitating contact between suitable partners. It is probably more important as a means of doubling the egg output from the population (Maynard Smith 1970). The expenditure of energy in maintaining two

sets of reproductive apparatus per individual is alleviated by the sharing of oogenesis and spermatogenesis in the same follicles, and the utilization of a common hermaphrodite duct, albeit an archaic condition (Morton 1955).

The temporal relationship of oogenesis to spermatogenesis in *A. crenata* was found to be intermediate between protandrous sexual succession and synchronous ripening. Sperm production reached a peak two months before maximum oocyte development, but small oocytes appeared during early spermatogenesis. This is similar to the condition described for some other archaic pulmonates (Morton 1955). Self fertilisation in the same follicle is presumably minimised by the germinal epithelium separating spermatids from developing oocytes, and the release of oocytes after sperm discharge from the follicle.

Whereas *Amphibola* has a long breeding life, another pulmonate of similar body size is *Lymnaea stagnalis* which shows another strategy, and begins breeding in its second year at 20mm shell length and has a two year breeding span (Berrie 1966). Follicle size increased with shell size in *A. crenata* and no reduction in sperm and oocyte production or other evidence of senescence with increasing size and age was observed. Mortality of large individuals during winter occurred, which is common amongst molluscs (Comfort 1957) and may possibly be caused by post-reproductive or climatic stress.

Gonad size increased by 100 to 200 percent during the breeding season. The visceral coil is tightly restricted within the shell which prevents a large increase in coil volume during gonad development. Gonad therefore increases mainly at the expense of digestive gland and the highly vacuolated interfollicle cells which

became reduced during breeding. The presence and reduction of these inter-follicle cells during development of the sex cells has been described in a wide variety of molluscs (e.g. Lammens 1967). Proportional increase in gonad was matched by the increase in follicle size, indicating that seasonal gonad development was a process of follicle size increase rather than follicle proliferation. Follicle number increased, however, with increase in size of animal.

The populations examined in the Avon-Heathcote Estuary appeared to have a prolonged breeding season as reproductive products were discharged periodically from each individual over a period of at least four months from Nov. to Mar. The season at the end of 1976 was predicted to produce more reproductive products than the previous season. Oocyte production was partially reinitiated in Apr. and May, but the winter was a period of degeneration and phagocytosis with spermatogenesis reaching a peak in Sep. Oocyte development may be delayed because of the slower maturation process required to build up yolk reserves, and thereby allowing complete discharge of the sperm first. Comparison of theduration of this breeding period (Nov. to Mar.) with that observed by Briggs (1972) in Northland (Aug. to May) suggests that climatic factors either directly or indirectly, through a factor such as food supply, are important in determing onset and termination of the breeding season. The beginning and duration of spawning for a particular population, have been related to weather and temperature for that locality in other molluscs (Lammens 1967).

No significant differences in gonad development, follicle and oocyte size, or sperm production, were identified between stations 1, 2, 3 and 4. A number of studies have looked at the

effect of pollutants on embryonic development (e.g. Calabrese and Nelson 1974) but no studies have been found to relate changes in gross morphology of gonad to an effect of pollution. Gamma irradiation has been shown to interrupt gonad maturation (Joosse, Boer and Cornelisse 1968) but Tripp (1974) was unable to detect cellular changes in gonad, gill, intestine and digestive and mantle tissue of oysters subjected to organophosphate insecticides. Infertility in the freshwater pulmonate, *Biomphalaria glabrata*, was induced by 0.1 ppm of cadmium and copper and fecundity was severely affected by chromium (Ravera 1977). Comparatively low levels of various organo chlorine insecticides appeared to increase the production of egg cases by gastropods (Eisler 1970) but changes in numbers of eggs in individual cases, or subsequent hatching success was not examined. In the present study the number of nidi laid per adult was highest at station 1 adjacent to the sewage outfalls (Chapter 4). This is considered to result from the large average size of individuals at station 1 rather from any stimulatory effect of conditions relating to the sewage outfall. It is possible that subsequent hatching rates varied for nidi at the different stations, but this was not investigated.

Larval trematode infection of digestive gland and gonad has been described for a number of molluscs and parasitic castration by mechanical disruption, and physiological suppression is common in a small proportion of the population of many molluscs (Malek and Cheng 1974). Incidence of the trematode appeared to conform to a general pattern of infection in *A. crenata* and possible stress from sewage pollution did not seem to predispose the populations at stations 1 and 2 to more chronic infection.

CHAPTER VI

LEVELS AND TOXICITY OF SOME HEAVY METALS

1. INTRODUCTION

Heavy metals have been identified as ubiquitous and particularly toxic components of industrial wastes (Merlini 1971). After discharge into a receiving water,metals and metallic salts are often accumulated by sedimentary processes (Gross 1970). The estuarine environment is a sink for minor elements arising both naturally from the geology of the catchment and as contamination from artificial wastes (Huggett et al 1973). The degree of accumulation of heavy metals from wastes in an estuary must therefore be considered against natural background levels of those elements.

Filter-feeding organisms, such as bivalves, have a marked propensity for the selective concentration of chemical materials from the aqueous medium into their tissues against the concentration gradient (Pringle et al 1968). Concentrations up to many hundreds of times the level of the environment have been recorded in these shellfish (Brooks and Rumsby 1965). This ability of shellfish to further concentrate substances which are present at artificially high concentrations from pollution, has lead to a growing number of studies of metal levels in consumable species as

they may constitute a hazard to human health (Nielsen and Nathan 1975). Some mollusc species have been used to monitor potentially toxic substances in coastal environments (Huggett et al 1973). Accumulation through a producer-herbivore system also has been described in the terrestrial pulmonate gastropod, *Helix aspersa* (Coughtrey and Martin 1977). Early studies concentrated on describing short-term (48 or 96h) lethal concentrations of individual substances particularly for freshwater fish (Doudoroff and Katz 1953). Laboratory studies into toxicity have been concerned normally with the control of one variate while keeping all other factors constant. Vernberg and Vernberg (1975) used different combinations of factors, such as temperature, salinity and oxygen to examine possibilities of their synergistic or antagonistic interactions in the environment. Recent work on the effects of heavy metals on molluscs has increasingly combined data on mortality with a consideration of sub-lethal effects on physiological processes such as respiration and ionic regulation (Brown and Newell 1972, Scott and Major 1972), behaviour (MacInnes and Thurberg 1973) and effects on fertilised eggs, embryos and larval stages (Okubo and Okubo 1962, Calabrese 1972). Studies at the levels of individual organisms, tissues and cells have been directed towards understanding the processes and dynamics of uptake, transport, storage, mode of toxicity, detoxification and physiological effects of heavy metals (e.g. Spronk et al 1973, Betzer and Pilson 1974).

The present study examined the levels of some heavy metals in the two study areas of the Avon-Heathcote Estuary (Fig. 6.1.) and determined the general ability of *Amphibola crenata* to withstand different concentrations of copper. The influence of

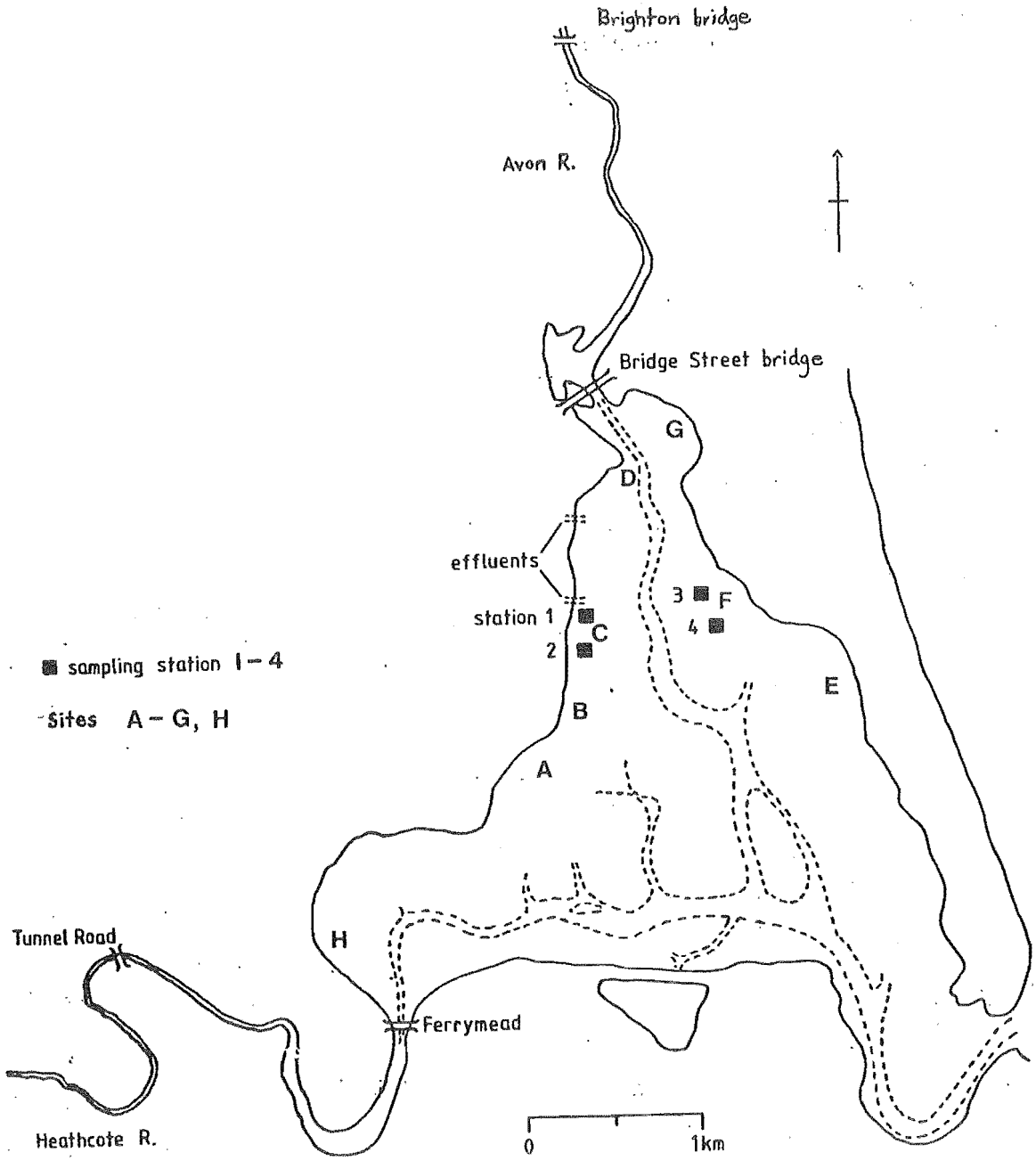


Fig.6.1. The Avon Heathcote Estuary showing the two main study areas stations 1 and 2, and stations 3 and 4), sites A to G, and the lower reaches of Avon River, from which samples were collected for heavy metal analysis. *Amphibola crenata* were collected at site H for toxicity experiments.

salinity, temperature, body size, and stage of life history on copper toxicity were determined in relatively long term (up to 24 days) studies of mortality in the laboratory. The choice of heavy metals, and in particular copper, was made arbitrarily to some extent, from the diverse range of possible industrial and domestic toxic contaminants in effluent from the sewage outfalls in the Avon-Heathcote Estuary. However copper was found to be present in *Amphibola* in a concentration which was higher than that of any other metal (see Results section of this chapter). There are many other studies on copper in molluscs and other estuarine invertebrates (e.g. Scott and Major 1972, Jones 1973, 1975a, b). There is therefore a considerable amount of data with which the results of this study can be compared.

Copper is recognised as one of twenty components commonly found in industrial wastes (Patrick et al 1968), arising from copper bearing water pipes, metal-plating wastes, and horticultural run-off. Copper occurs naturally at about 0.003ppm in seawater (Goldberg 1965), but up to 0.1 to 1.0ppm naturally in gastropods, depending on the season (Betzer and Pilson 1974). Although copper in low concentrations is essential to molluscs for enzyme and respiratory functions, for example cytochrome c-oxidase and as a constituent of haemocyanin (Spronk et al 1973) the optimum range is narrow. Above certain concentrations it becomes one of the most toxic of the heavy metals (Bowen 1966). Its toxicity is dependent on factors such as temperature, pH, the presence of other metals and salts, and the susceptibility of particular species. For example, copper is ten times more toxic to the soft-shell clam than to the hard shell clam (Pringle et al 1968).

Estuarine organisms are adapted to cope with changes in environmental factors such as osmotic and ionic concentration and exposure to temperature and to desiccation stress. This high degree of adaptation to potentially stressful conditions may predispose an estuarine species towards coping with stress imposed by changes in particular ions in the food chain or surrounding water. On the other hand a high degree of adaptation may reduce a species' flexibility to withstand abnormal conditions. The mechanisms which are required for survival in the normal range of estuarine conditions are particularly vulnerable to inhibition or interference by toxins which affect enzyme systems (Vernberg and Vernberg 1972, Jones 1973). The pulmonate lung allows *Amphibola* to exploit rich organic conditions where high pollutant levels may occur in the water and sediment. At the same time its burrowing habit, large internal reservoir (Chapter 3) and operculum may allow it to temporarily avoid toxic conditions.

Amphibola possess many of the advantages described by Ravera (1977) for the freshwater prosobranch, *Biomphalaria glabrata* for studying relatively long-term responses to pollutants in the laboratory. *Amphibola* can be maintained in a small closed system with a minimum of requirements for survival. Disadvantages with *Amphibola* are that the extent to which retraction of the operculum may reduce diffusion from the external medium is unknown; and the effects which continuous immersion may have on its normal physiology are also unknown. Laboratory studies of uptake of heavy metals from sediment are difficult to interpret because of the problem of determining the extent to which metal ions become bonded to the sediment, and the proportion of uptake through ingestion of organically-bound metal as compared with

direct diffusion and uptake of free ions through the foot and shell. In the present study, toxicity of copper to *Amphibola* was studied using an aqueous solution of the copper with regular removal of mucus and faecal material which can bind metal ions (Scott and Major 1972).

11 METHODS

(1) Field Samples

(i) Collection

Two samples, each of 8 *Amphibola* larger than 15mm shell length were collected at each station, 1 to 4 (Fig. 6.1.), on 5 Jun. and 21 Jun. 1974. Two sediment samples of approximately 10g dry weight were collected from the upper 10mm of sediment at each station on 5 Jun. and three similar samples were collected from the sediment surface at sites A to G (Fig. 6.1.) on 5 Jul. 1974. Sites C and F were located between stations 1 and 2, and stations 3 and 4 respectively. Eighteen sediment samples were collected randomly along the Avon River bank between Bridge Street bridge and Brighton bridge (Fig. 6.1.) on 10 Jul. 1974, to compare metal levels in the silty sediments of the lower reaches of the Avon River with those in the more sandy sediments of the estuary, represented by sites A to G.

(ii) Analysis

Animals were kept for 24h at 15°C after collection to eliminate sediment from their alimentary canals. The soft bodies of each sample of 8 individual *Amphibola* were removed from their shells, the opercula cut off, and the soft body sample weighing 7 to 12g placed in a pre-weighed porcelain crucible. Wet weight was measured to 0.01g on an electric balance and then dry weight

was measured after animals had been dried at 65°C for 72h in an oven ventilated with a fan. Each sediment sample was spread in a thin layer around the inside of a porcelain crucible, and dried as for soft body samples. Each sample in its crucible was then ashed for 12h at 450°C following the method described by Nielson and Nathan (1975). The sediment digestion combined free forms of the metals with that released by digestion of the organic fraction. Metals in the ash were dissolved in 10ml 3M nitric acid (analar grade) and analysed by atomic absorption spectrophotometry. The analyses were carried out with the assistance of Nielson and Nathan who determined that the percentage recoveries by this method for different metals were: cadmium 100 ± 2%, zinc 99 ± 2%, lead 93 ± 4%, and copper 100 ± 1%.

Empty crucibles were taken through the analysis to determine contamination from equipment and materials, which was found to be negligible. All equipment and containers used for collecting, preparing and analysing samples, was non-metallic or of stainless steel. Glassware and crucibles were washed with analar acid, followed by several rinses in glass-distilled water, between each sample.

(2) Laboratory Studies

(i) Post-larval *Amphibola*

The survival was investigated of *Amphibola* larger than 15mm shell length, exposed to copper (II) concentrations of 2.5, 5, 7.5, and 10ppm, and controls without added copper. The effect of salinity and temperature on copper toxicity was examined by setting up test concentrations of copper at 15°C in salinities of 2, 4, 6, 8 and 100% seawater, and in 4% seawater at temperatures of 10, 15 and 20°C. Mortality was compared in animals of less

than 10mm, 10 to 15mm, and greater than 15mm shell length in different concentrations of copper, at 15°C, in 10% seawater. The mean dry weights of the first five *Amphibola* to die at each level of copper in 4% seawater at 10, 15 and 20°C, was compared with the mean weights of the remaining five which survived beyond the 50% lethal concentration time (LT_{50}). Toxicity of the copper test concentrations was compared at 20°C in 25% seawater with that of solutions containing 10, 20, 30, 40 and 50ppm (II). The ability to survive in water of different salinities without added copper, after brief exposure to high copper concentrations, was also investigated. Different groups of animals were exposed to 10, 25, 50 and 100ppm copper for 12, 24, 48 and 72h. Each group was then removed from its treatment level, rinsed in water with the salinity of the subsequent test solution, and survival tested in salinities of 2% and 10% seawater at 15°C

Amphibola were collected immediately prior to the experiment from above M.W.L. on the southwest slopes of the estuary in area H (Fig. 6.1.) where *Amphibola* occurred in all sizes of shell. The experiments were carried out during May, Jun. and Jul.1976 and all animals were therefore winter acclimated. After collection, animals were washed with 10% seawater and divided randomly into groups of 10 individuals. Each group was placed into 500ml glass jar containing 20ml of experimental solution and each test level was duplicated. The copper and zinc concentration series were prepared daily from stock solutions of the metal (II) chloride salt dissolved in distilled water. Experimental metal levels were chosen after a number of preliminary series, to fit an experiment duration of 24 days. The seawater series were prepared by diluting filtered seawater (37.4%) with aerated distilled

water. There was no visible precipation of metal in the experimental media.

The test containers were covered circular glass jars 100mm deep by 100mm diameter with a raised dimple in the bottom which reduced the test solution depth to about 2mm in the central area of the container. With this provision animals of shell length larger than 10mm could breathe with the lung but the foot remained immersed in the medium. Small animals (less than 10mm shell length) frequently crawled out of the medium up the wall of the jar. All animals were removed daily and checked for survival. An animal was considered to be dead if it failed to retract after mechanical stimulation of the operculum. After this check the test container was rinsed with water of the test salinity to remove particulate matter and mucus, and a fresh solution added. In this way changes in metal ion concentration by adsorption were reduced. Food was not provided during the course of an experiment. The test jars were kept in constant environment cabinets with a lighting cycle maintained according to external day length, and the temperature controlled at 10, 15, or 20°C.

(ii) Eggs and larval *Amphibola*

Two nidi were collected immediately after being laid on the shore near station 3 (Fig. 6.1.) on 24 Apr. 1976, at the end of the breeding season, and separated into small glass petri dishes. Replicate dishes, using a part of each of the nidi, were prepared for each of the following treatments: 100, 25, 10, 7.5 and 5% seawater without added metal, and 2.5, 5. 7.5 and 10ppm copper in 25% seawater. The dishes were covered, placed in a constant environment cabinet at 15°C, with external day length, and removed for a few minutes each day to provide fresh medium and to examine

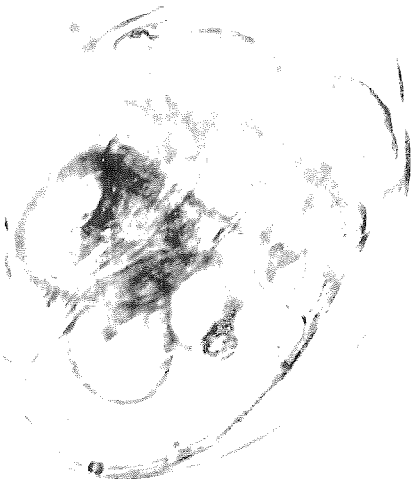
Photographs on facing page.

Fig.6.2a. The egg several hours after being laid, when the experimental treatments were set up. A developing blastula is contained within a gelatinous capsule surrounded by an egg-shell membrane. The egg-shell membrane is indistinct in this photograph but can be seen. Sand particles, separated from the nidus, surround the egg.

Fig.6.2b. Embryo at the veliger stage on the 8th day after nidus formation, within the shell membrane. The beating cilia on the velum can be seen at the top of the veliger. The shell encloses the visceral mass oriented to the bottom of the photograph.

Fig.6.2c. Veliger larva stage soon after hatching from the egg capsule on the 11th day after being laid. The operculum appears as a line below the velum.

a



b

c

hatching success under a binocular microscope (Fig. 6.2a., b.).

Eggs were hatched in 25% seawater, without added copper, to provide free-swimming veligers (Fig. 6.2c.) which were placed in small glass syracuse dishes containing 0.1, 0.5, and 1ppm copper and controls in 25% seawater. Each level was duplicated and dishes were checked for veliger survival each 2h during the first 8h, and at 8h intervals thereafter. Before checking for survival distilled water was added to the test solution to bring it to the initial volume, aerate the medium and stimulate activity in veligers which had settled at the interface between the side of the dish and the meniscus. Apparently 'dead' veligers sometimes revived after the addition of a drop of distilled water. Activity was observed under a microscope and veligers were considered to be dead when the cilia on the velum ceased to beat and the veliger settled to the bottom of the container. The medium was not changed and nutrients were not provided during the experiment which lasted for 7 days. Between observations dishes were placed in a constant environment cabinet at 15°C and external day length.

III RESULTS

(1) Field Samples

The metals in *Amphibola* were found in the same order of increasing concentration at each of the stations on 5 and 21 Jun. 1974. Concentrations increased from cadmium, the lowest, to lead, cobalt, nickel, zinc and copper, which was highest, at 64 to 88ppm per g dry weight (Table 6.1). *Amphibola* from stations 1 and 2 had higher levels of nickel per unit dry weight, and of copper on one occasion, than those from stations 3 and 4. For all other metals

Table 6.1 Mean levels of some heavy metals in the soft tissue of *Amphibola crenata*, and surface sediment collected from stations 1, 2, 3 and 4, and concentration factors from sediment to soft tissue.
Data are combined for stations 1 and 2 (S12) as well as for stations 3 and 4 (S34). Concentrations are given as parts per million per gram dry weight (ppm/g dw), or nanograms of metal per individual (ng/i).

	Amphibola crenata soft tissue				Sediment	
	ppm/g dw		ng/i		ppm/g dw	
	(S12)	(S34)	(S12)	(S34)	(S12)	(S34)
5 Jun.						
Mean dry wt(g)	0.36	0.21	0.36	0.21		
cadmium	0.87	1.22	0.31	0.25	0.17	0.11
cobalt	8.97	11.85	3.25	2.34	1.57	0.72
copper	74.69	65.32	26.87	13.44	3.39	1.59
lead	6.46	8.13	2.31	1.69	8.81	4.82
nickel	19.55	12.95	7.10	2.57	3.02	1.23
zinc	57.14	75.64	20.63	15.63	29.09	15.94
21 Jun.						
Mean dry wt(g)	0.34	0.19	0.34	0.19		
cadmium	0.77	1.47	0.26	0.27		
cobalt	9.25	13.05	3.16	2.41		
copper	64.56	88.36	22.51	16.57		
lead	7.44	9.42	2.57	1.76		
nickel	16.76	14.19	5.78	2.66		
zinc	59.74	67.15	20.79	12.51		

Concentration Factor (c) (ppm/g dw soft tissue ÷ ppm/g dw sediment)
mean dry wt (S34)
÷ mean dry wt (S12)
= 1.7

	C1 (S12)	C2 (S34)	C1÷C2
cadmium	4.1	13.6	3.3
cobalt	5.0	6.0	1.2
copper	54.8	101.1	1.8
lead	0.7	1.6	2.3
nickel	4.1	5.0	1.2
zinc	1.5	3.1	2.1

concentration per unit dry weight was higher in *Amphibola* from stations 3 and 4. Amount of metal per individual animal, however, was higher at stations 1 and 2 because of the higher individual average weight of animals from these stations. Nickel and copper showed the highest proportional difference in amount of metal per individual animal between the two areas.

The order of concentration of metal per g dry weight of sediment was the same at all stations (1 to 4) from cadmium, the lowest, to cobalt, nickel, copper, lead and zinc (Table 6.I). Thus the order was different from that in *Amphibola*, with copper concentration being relatively lower in sediment. For all metals concentrations in the sediment at stations 1 and 2 were approximately twice those at stations 3 and 4. The concentration factor from sediment to *Amphibola* tissue dry weight was found to be highest for copper (54.8 times at stations 1 and 2, 101.1 times at stations 3 and 4) (Table 6.I). Lead showed a concentration factor of less than 1 at stations 1 and 2; that is, a higher concentration of lead was bound in the sediment than was taken up in *Amphibola* tissue.

Metal concentrations in sediment in the north west segment of the estuary showed a displacement of copper in the order of concentration down to second lowest after cadmium (Table 6.II). The highest concentrations of each metal occurred at site D, and site G at the point where the Avon River widened onto the estuary mud flats, and adjacent to an early rubbish dump site. The order in sediments in the lower Avon River, sampled on 10 Jul., was the same as for sediment sampled at stations 1 to 4 during Jun. 1974 (Table 6.II). One extremely high concentration of lead was recorded near to a drain. If this record were included, lead would

Table 6.11. Mean levels of some heavy metals in surface sediment collected in the north west segment of the Avon-Heathcote Estuary and the lower reaches of the Avon River.

Metal concentrations in ppm per gram dry weight of sediment.

Station	A	B	C (between stns1,2)	D	E	F (between stns3,4)	G
cadmium	0.09	0.15	0.20	0.44	0.10	0.10	0.12
cobalt	1.72	2.16	1.84		1.36	2.08	3.77
copper	0.84	0.98	1.27	4.44	0.76	0.72	1.82
lead	6.49	9.04	9.89	19.81	4.45	5.38	11.84
nickel	2.18	2.77	4.45	7.03	2.52	2.74	6.04
zinc	19.99	24.79	37.96	80.12	19.68	22.78	40.40

Metal levels in 18 samples of sediment taken in the lower Avon River between Bridge St. Bridge and Brighton Bridge.

	Range ppm/g dw sediment	Mean ppm/g dw sediment
cadmium	0.11 - 0.15	0.20
cobalt	1.20 - 4.47	2.84
copper	1.20 - 24.02	5.99
lead	7.58 - 268.10	59.14 (17.35 if one extremely high value is removed)
nickel	1.71 - 8.40	4.84
zinc	24.06 - 100.41	56.37

be the metal with the highest concentration in the Avon River. The levels on the river banks of each of the metals were found to show a very wide range depending on the type of substrate (from coarse sand and gravel to fine silt) and the proximity of drains and rubbish dumps. Zinc occurred at the highest concentration in all but one of the sediment samples from stations 1 to 4, sites A to G, and the lower Avon River (Fig. 6.1.).

(2) Laboratory studies

Cumulative per cent mortality with time (days) was plotted for each treatment level; for example, each metal concentration at each level of salinity (Fig. 6.3.). From each curve the 50% lethal concentration time (LT_{50}) in days for a treatment was calculated. The LT_{50} were then plotted (for example, Fig. 6.4.) for direct comparison between the effects of different combinations of levels of both metal concentration and other factors. In these results, unless otherwise stated, adults refers to animals with a shell length greater than 15mm.

(i) Salinity

Decreasing salinity below 8% seawater, without added copper, caused an increase in adult mortality (Fig. 6.3.). Time until the first death was reduced from 19 days in 8% seawater, to 4 days in 2% seawater. All adults were dead in 2% seawater within 11 days. At4% and 6% seawater the first deaths occurred after 7 days, but there was considerable variation between animals in survival time, particularly after LT_{50} (Fig. 6.4.) when either more resistant animals remained, or remaining animals had acclimated to a lower salinity. Increase in salinity from 10% to 100% seawater did not affect adult mortality and at 100% seawater LT_{50} was not reached

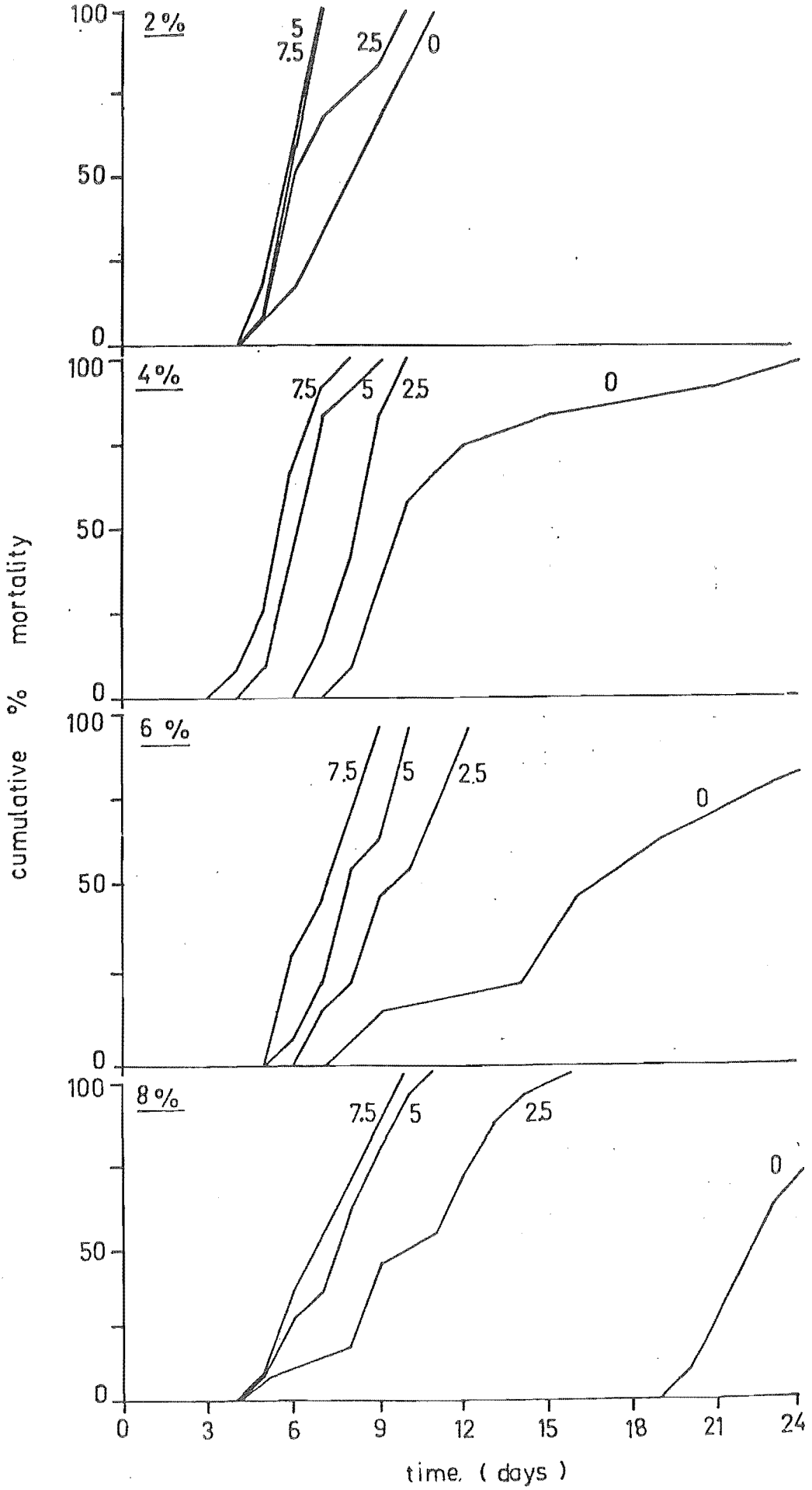


Fig.6.3. Cumulative per cent mortality of *Amphibola crenata* in controls without added copper, and in 2.5, 5, and 7.5ppm copper(II) at salinity levels (from top) of 2%,4%,6% and 8% seawater.

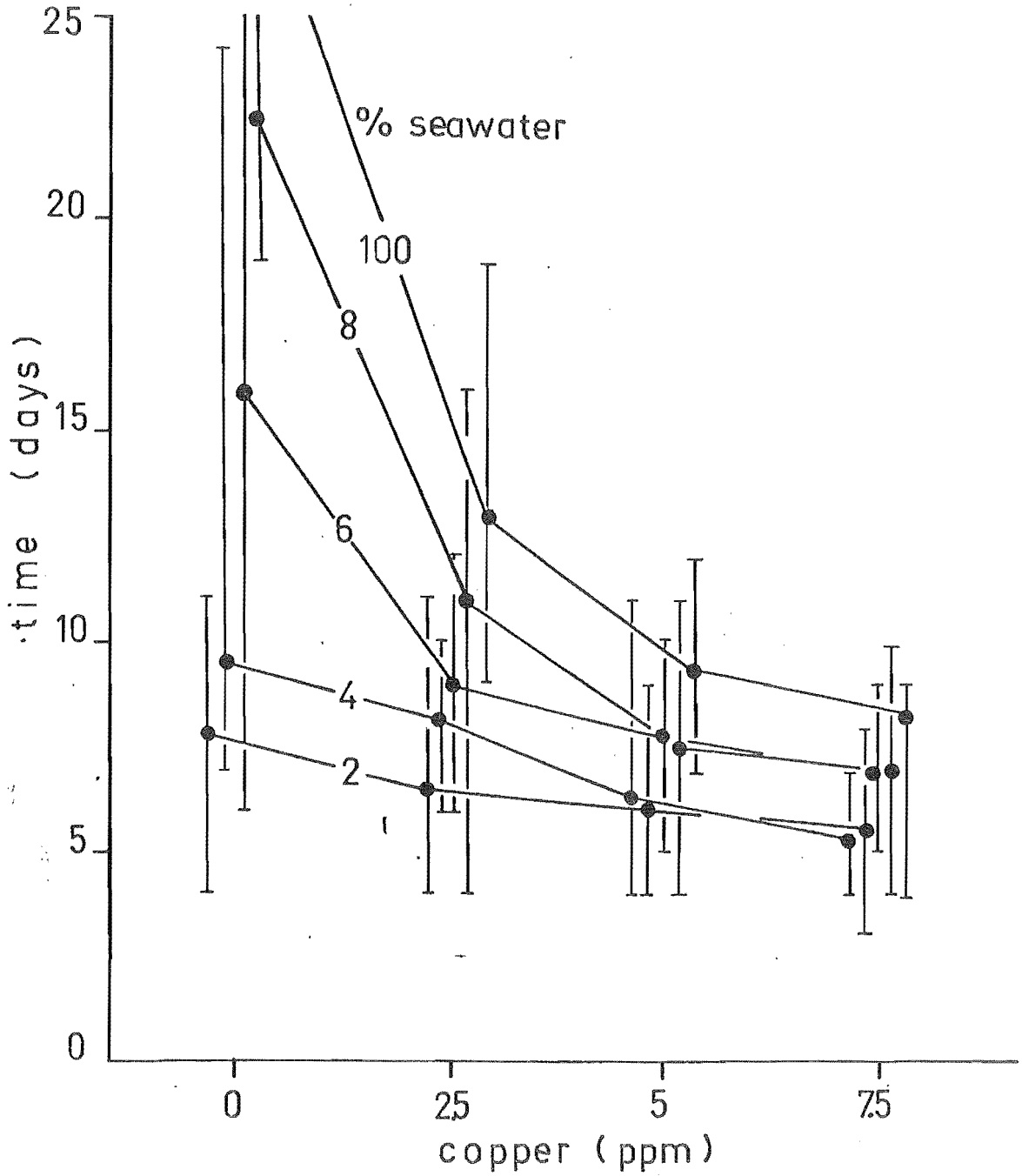


Fig.6.4 Time taken for 50% cumulative mortality (LT_{50}) of *Amphibola crenata* adults in controls without added copper, and in 2.5,5,and 7.5ppm copper (II) at salinity levels of 2%, 4%, 6%, 8%, and 100% seawater at 15°C. Range of survival times for individuals in each treatment are shown by vertical lines.

in 24 days.

The addition of up to 7.5ppm copper had relatively little effect on the time to the first death, with all animals surviving at least three days. The presence of metal induced mucus production, which formed a coagulant in the experimental media. In 2% seawater all animals died within 7 days of each other, whereas at higher salinities the range of individual survival times was greater for each level of metal concentration. In 6% and 8% seawater there was considerable difference in survival time between media without added copper and in 2.5ppm copper, but at all salinity levels the difference in survival times between 2.5, 5 and 7.5ppm copper was relatively small (Fig. 6.3.). With increasing copper concentration the difference between survival times for each salinity was reduced to approach a LT_{50} plateau of about 6 days, independent of salinity value.

(ii) Temperature

The most noticeable effect of temperature on mortality was that an increase in temperature from 15 to 20°C markedly reduced the range of individual survival times when there was no copper present (Fig. 6.5.). For each level of added copper from 2.5 to 7.5ppm there was a slight decrease in LT_{50} and the range of survival times, with increase in temperature, up to 20°C.

The 50% survival contours in days show that for combinations of copper concentration and temperature there was a toxicity threshold above 2.5ppm copper and 10°C which produced a marked change in rate of decrease in survival time from 13 to 9 days (Fig. 6.6a.). This threshold for combinations of copper and salinity occurred above 2.5ppm copper and below 8% seawater to produce a similar decrease in survival time from 16 to 11 days

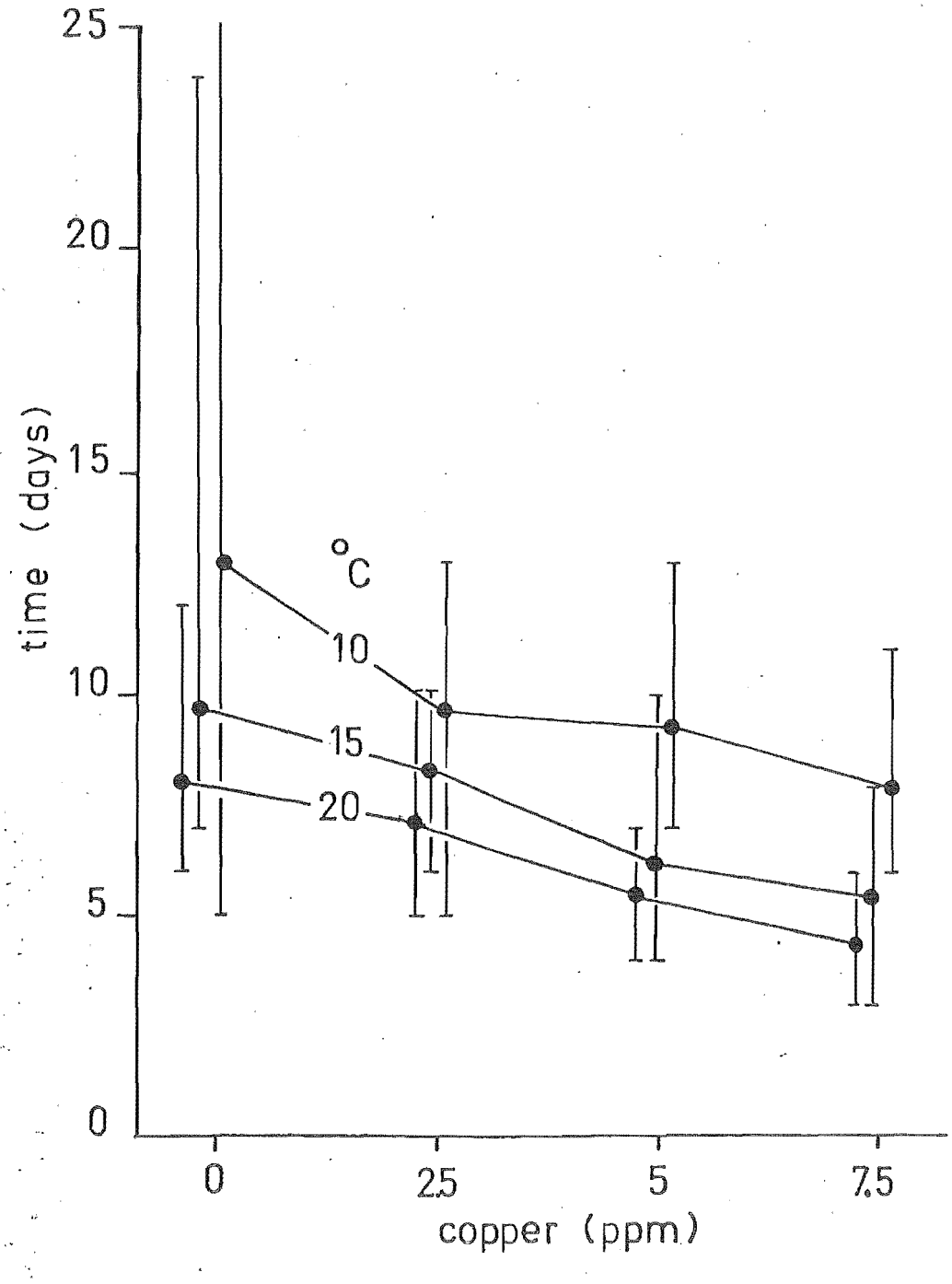
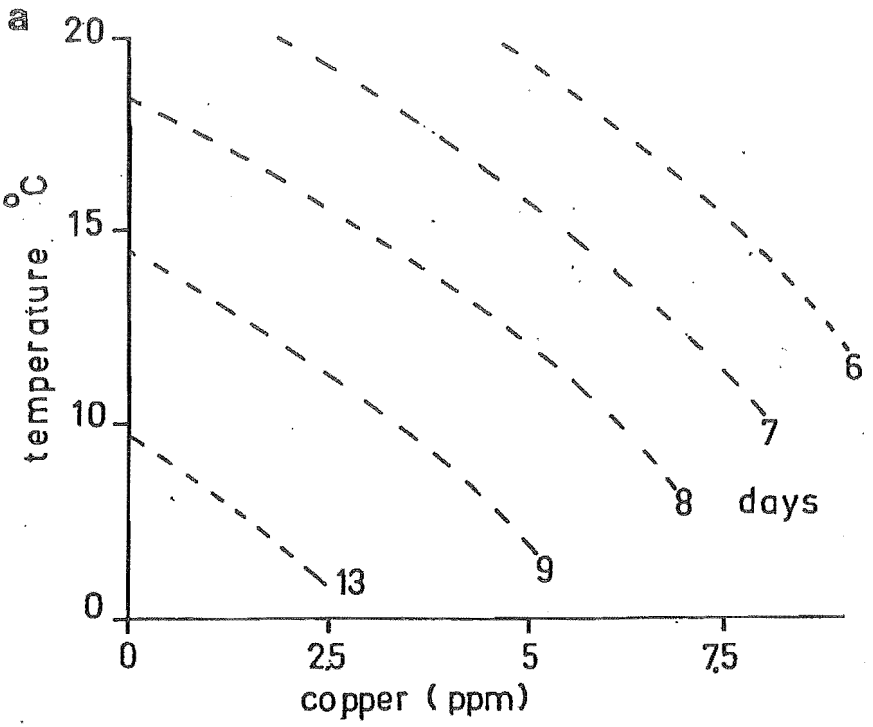
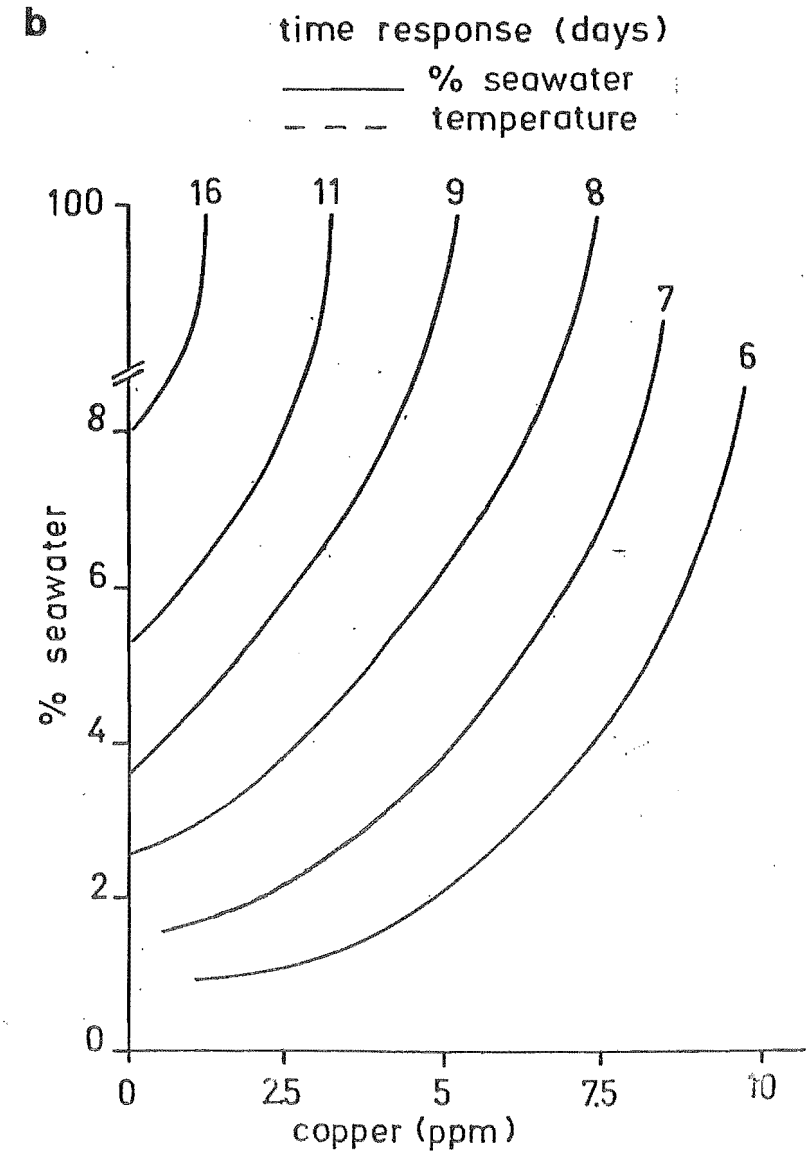


Fig.6.5. Time taken for 50% cumulative mortality (LT_{50}) of *Amphibola crenata* adults in controls without added copper, and in 2.5, 5 and 7.5ppm copper(II) at temperatures of 10, 15 and 20°C in 4% seawater. Range of survival times for individuals in each treatment are shown by vertical lines.


a
20
15
10
0
°C
temperature
6
7
8
days
9
13
0
2.5
5
7.5
copper (ppm)


b
time response (days)
% seawater
temperature
100
16
11
9
8
7
6
8
6
4
2
0
% seawater
0
2.5
5
7.5
10
copper (ppm)

(Fig. 6.6b.).

(iii) Body size

Larger animals had much longer survival times than small animals under the experimental conditions in 10% seawater at 15°C with less than 2.5ppm added copper (Fig. 6.7.). No mortality occurred over 24 days at 1ppm copper for animals larger than 15mm. With an increase in copper concentration to 7.5ppm there was a reduction in the difference between different size classes in their survival times. At all copper concentrations LT_{50} was higher for larger animals than for smaller animals. The mortality of different sizes approached a LT_{50} plateau of about 3 days with increasing copper concentration. The range of individual survival times was similar for all 3 size classes at 2.5 to 7.5ppm copper, but at 1ppm the individual range was much smaller in animals less than 10mm shell length than for larger animals. In preliminary experiments it was shown that in seawater dilution series down to 0.5% seawater, without added copper, animals less than 10mm shell size had a much higher survival than larger individuals which showed a high mortality at 2% seawater.

There was no consistent relationship between size and survival time within *Amphibola* larger than 15mm shell length, when mean dry weights of the first *Amphibola* to die were compared with longer surviving individuals at different levels of copper at 10, 15 and 20°C in 4% seawater (Table 6.III). The two largest animals, with a dry weight (shell and soft body) over 3g, were amongst the first 5 animals (out of 10 per treatment level replicate) to die in their treatments. At 7.5ppm copper the mean weights of the first animals to die were higher than those of animals which survived longest at each temperature.

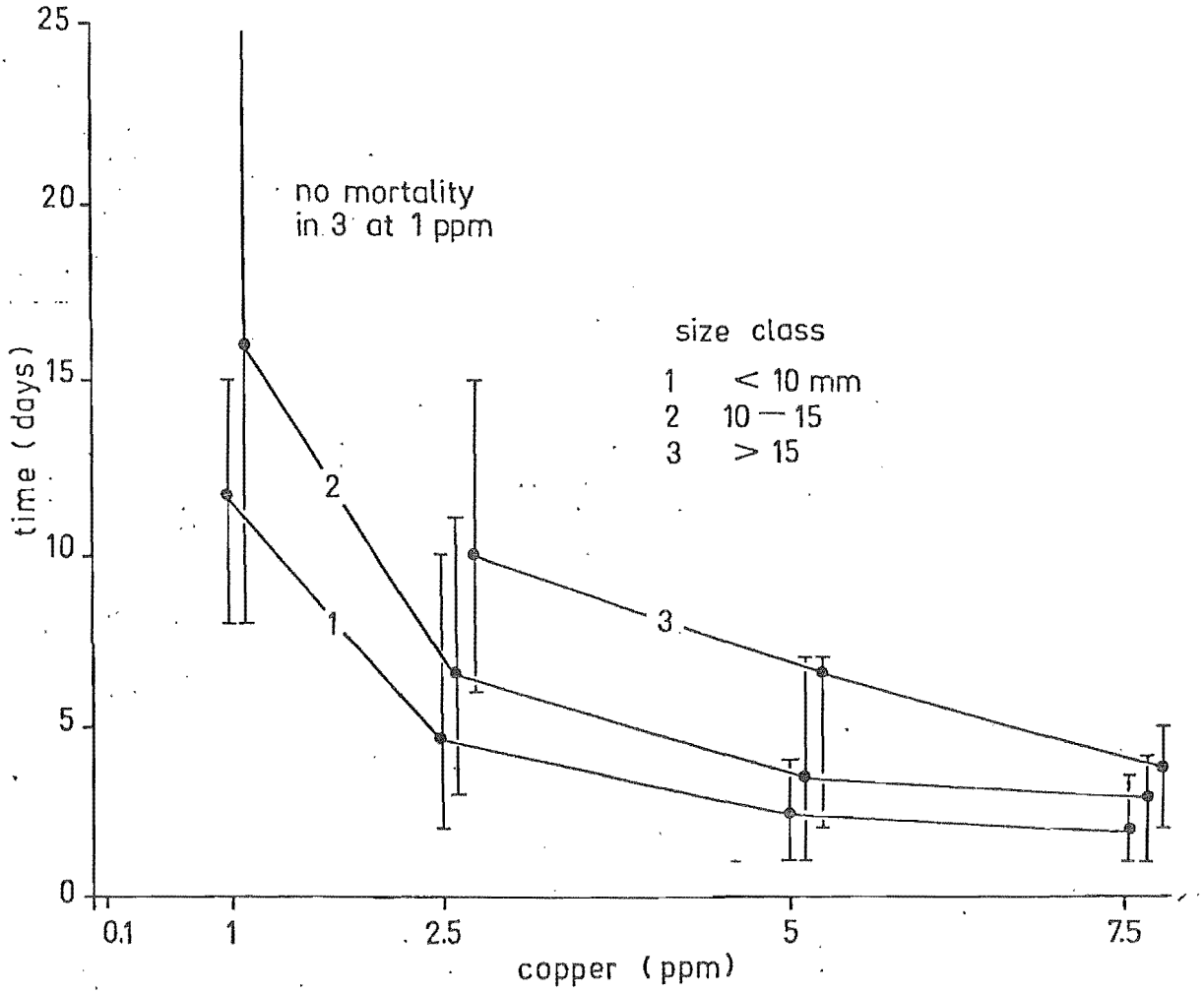


Fig.6.7. Time taken for 50% cumulative mortality (LT_{50}) of three different size classes of shell length of *Amphibola crenata* at 15°C in 10% seawater. Range of survival times for individuals in each treatment are shown by vertical lines.

Table 6.III. Comparison of mean dry weights of *Amphibola* dying prior to the 50% lethal concentration time, with weights of those dying after the 50% lethal concentration time. Treatments were different concentrations of copper at three different temperatures. Dry weight (g) includes shell and soft body weight.

Temperature	Copper conc.(ppm)	0	2.5	5	7.5
10°C	Prior	1.97	1.88	1.98	2.04(3.22)
	After	1.95	2.28	2.26	1.90
15°C	Prior	2.05	2.21(3.27)	2.01	1.91
	After	2.00	1.82	1.81	1.77
20°C	Prior	1.98	1.89	2.10	2.19
	After	2.04	2.39	2.02	1.94

Weights in parenthesis identify the two *Amphibola* used in the experiments which weighed more than 3 g.

(iv) Copper and zinc

Copper was approximately 4 times more toxic than zinc to adult *Amphibola* in 25% seawater (Fig. 6.8.). There was no mortality in 1ppm copper tested under these conditions. The curves for both copper and zinc were the same shape over the same proportional (4-fold) range of concentrations, and both metals produced the same decrease in variation in individual survival times with increasing metal concentration.

(v) Brief high toxicity exposure and subsequent survival with salinity stress

Amphibola exposed to high concentrations of copper, from 10 to 100ppm for 12 and 24h showed a marked difference in subsequent survival between 2% and 10% seawater (Fig. 6.9.). This difference between animals in their survival in 2% and 10% seawater was reduced as survival times decreased with increasing concentration of the copper solution need for the initial exposure. At 100ppm, survival times in both 2% and 10% seawater were low and there was overlap in the curves for each seawater level. Individual variation in survival times also decreased with increasing copper concentration and with decreasing salinity from 10% to 2% seawater. There was very little difference between the effect of 12 and 24h initial exposure except at 10ppm copper, the lowest concentration used in this experiment, which caused no mortality after 12h exposure until after 15 days in 10% seawater, as compared with mortality after 10 days in 10% seawater, after 24h exposure (Fig. 6.9.). An increase in time to 48h of initial high toxicity exposure produced a marked shift of the subsequent survival time curves by 2 to 3 days in 2% seawater, and by 5 to 15 days in 10% seawater (Fig. 6.10.). There was little difference

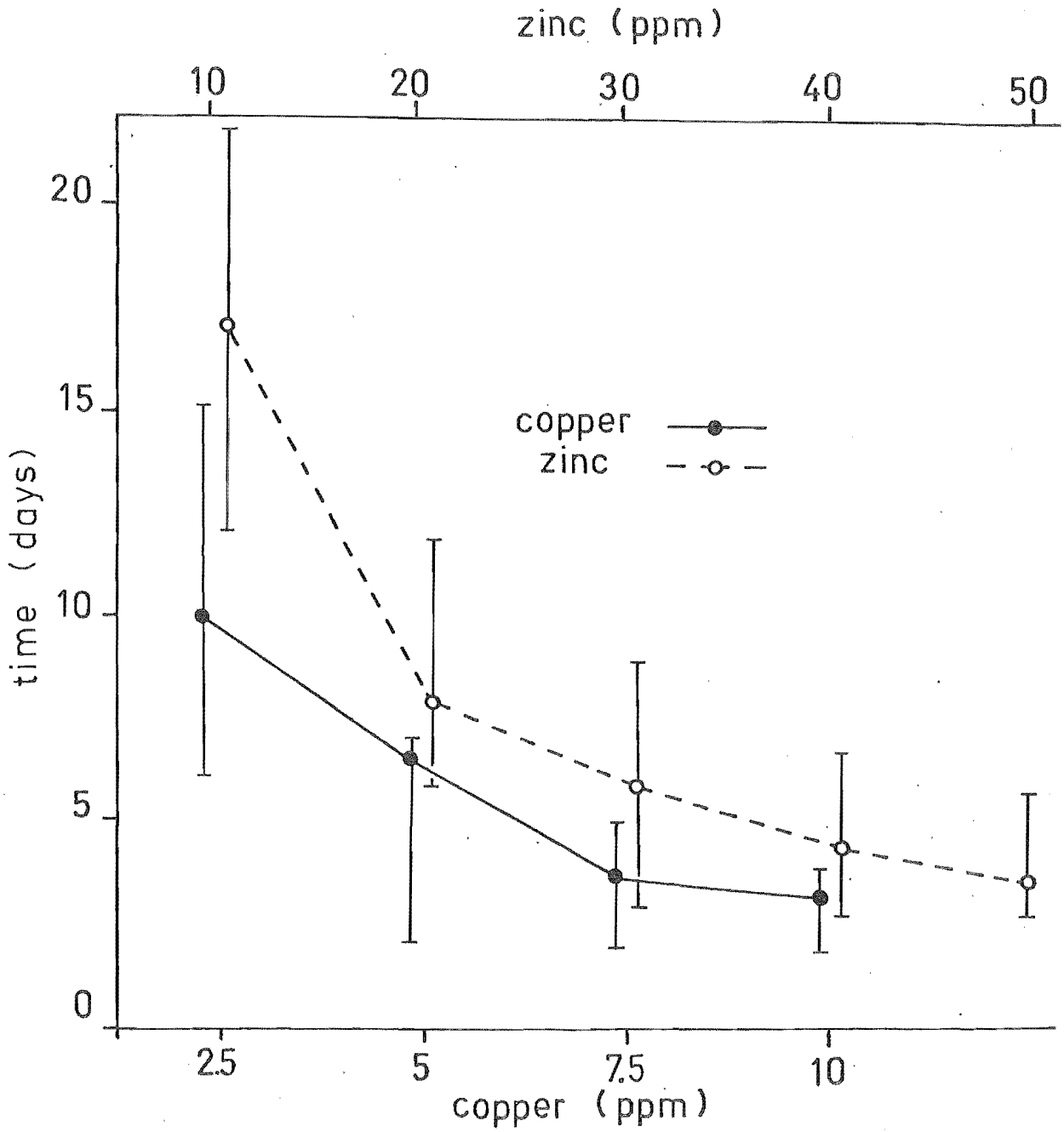


Fig.6.8. Times taken for 50% cumulative mortality (LT_{50}) of *Amphibola crenata* adults in different levels of copper (II) compared with LT_{50} in different levels of zinc (II) at 20°C in 25% seawater. Range of survival times for individuals in each treatment are shown by vertical lines.

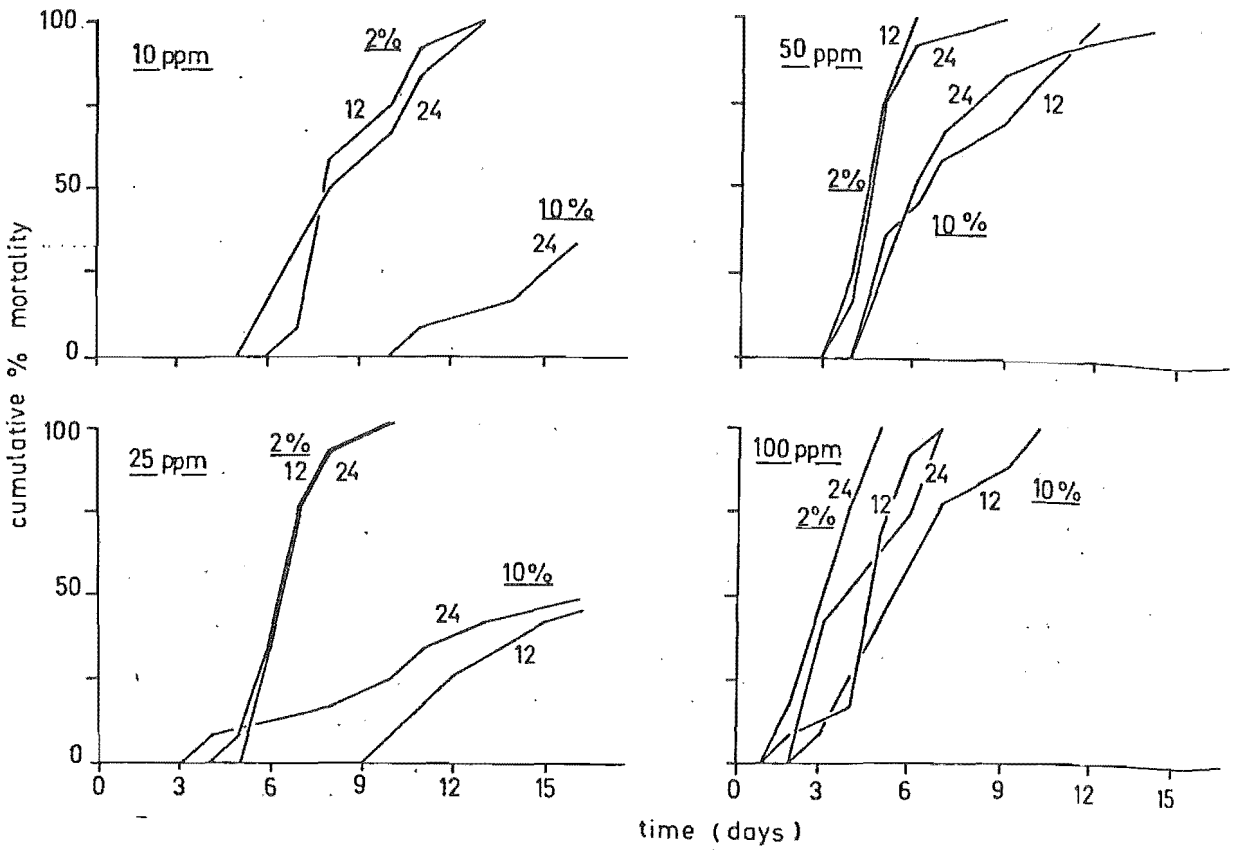
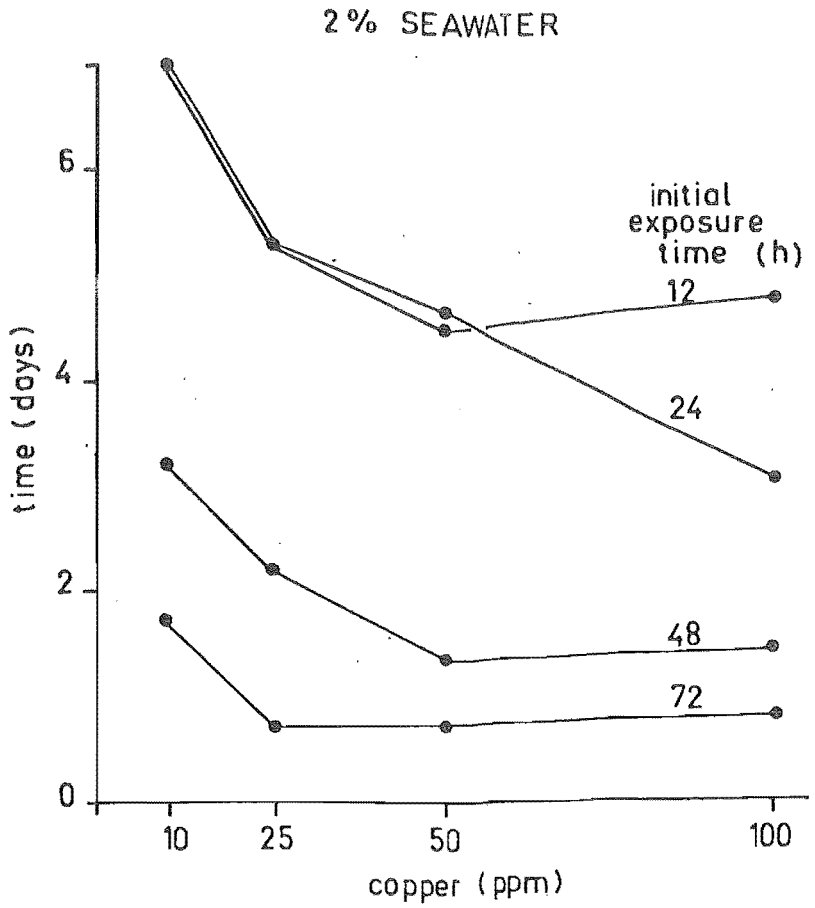
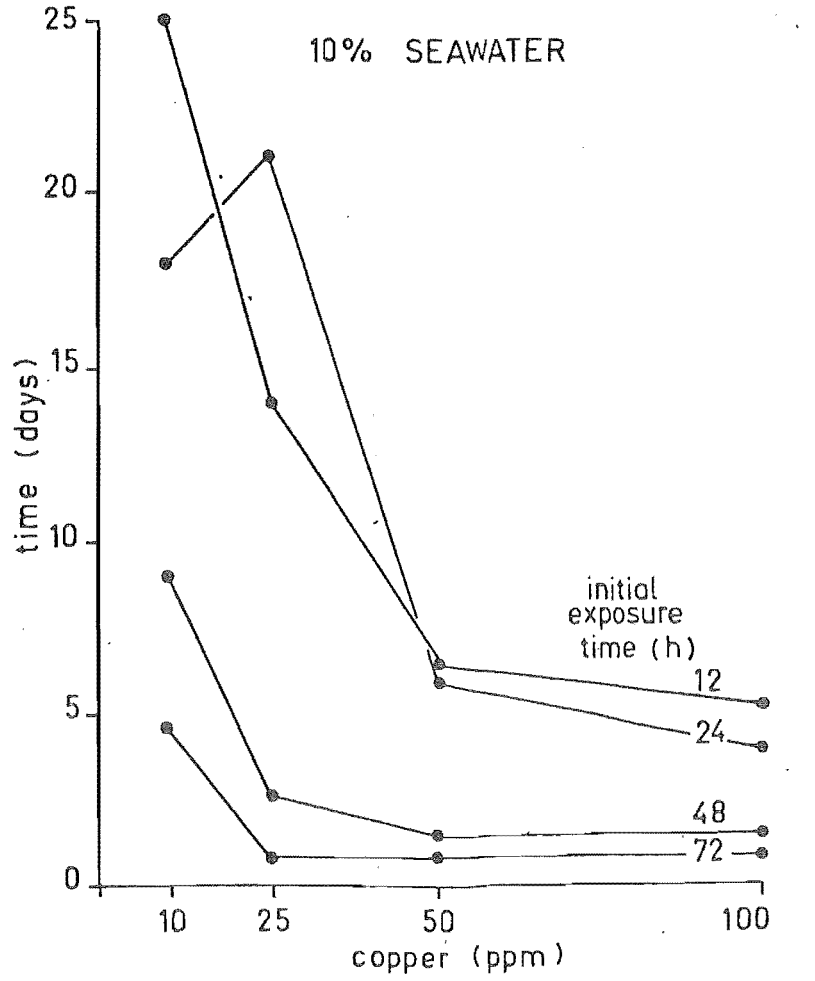


Fig.6.9. Cumulative per cent mortality of *Amphibola crenata* adults exposed to 2% and 10% seawater at 15°C after pretreatment in different copper concentrations (10,25,50 and 100ppm) for 12 and 24h.

Fig.6.10. (facing). Time taken for 50% cumulative mortality (LT_{50}) of *Amphibola crenata* adults exposed to 2% and 10% seawater at 15°C after pretreatment in different copper concentrations (10,25, 50 and 100ppm) for 12, 24, 48 and 78h.


2% SEAWATER
time (days)
6
4
2
0
initial
exposure
time (h)
12
24
48
72
10
25
50
100
copper (ppm)


10% SEAWATER
time (days)
25
20
15
10
5
0
initial
exposure
time (h)
12
24
48
72
10
25
50
100
copper (ppm)

in LT_{50} between 12 and 24h treatments after exposure to 50 and 100ppm copper, or between 48 and 72h treatments after exposure to 25, 50 and 100ppm. Thus lower initial exposure times and copper concentrations and lower subsequent stress produced a greater differential between survival times for each treatment level, but in all treatments exposure to copper for at least 12h eventually caused a delayed increase in mortality.

(vi) Egg development

Hatching success of *Amphibola* eggs was affected by both salinity and copper concentration with a marked drop in hatching percentage at salinities less than 10% seawater, and at copper concentrations greater than 2.5ppm. The early blastula stage of eggs exposed to lethal salinity or copper treatments became distorted and contracted. For eggs hatched in solutions with salinities above 10% seawater and copper concentrations below 2.5ppm there were no visible differences in general appearance and activity patterns of veligers after they were placed in 25% seawater without added copper, which would indicate sub-lethal changes. (Table 6.IV).

(vii) Veliger survival

There was a high veliger survival over 4 days in seawater concentrations above 3%, which was more dilute than media which interrupted egg development, but copper concentrations in which eggs developed were highly toxic to veligers. In copper concentrations over 0.5ppm, veligers produced large amounts of mucus which appeared to interfere with the movement of the cilia of the velum, and at 10ppm all veliger movement ceased within 5h exposure. (Table 6.V).

Table 6.IV Hatching success of eggs incubated in different salinities, and different concentrations of copper in 25% seawater.

Salinity (%seawater)	Hatching success(%)	Copper conc. (ppm)	Hatching success(%)
25	80 - 100	2.5	80 - 100
10	80 - 100	5	50
7.5	10	7.5	5
5	0	10	0
distilled water	0		

Table 6.V. Survival of *Amphibola* veligers at different salinities, and in different concentrations of copper in 25% seawater.

Salinity (%seawater)	Survival % after 1,2,3,4 days 1	2	3	4	Copper conc. (ppm)	Survival
6	100	90	90	80	0.1	24h-swimming, survived 5 days
4.5	85	80	80	80	0.5	24h - no movement
3	85	80	80	70	1.0	3h - mucus, 5h - no movement
1.5	70	0	0	0		

IV DISCUSSION

The difference between tissue levels of metal per unit dry weight, which were higher generally in the smaller *Amphibola* at stations 3 and 4, and values for the total of each metal per individual, which were higher at stations 1 and 2, demonstrates the difficulty of interpreting differences in tissue metal concentrations between populations. Other studies have shown that relationships between tissue concentration and body size may depend on the particular mollusc species (Boyden 1974), patterns of uptake into different organs, interactions between metal uptake rates, and the time of the year (Betzer and Pilson 1974, Coughtrey and Martin 1977). Bryan and Hummerstone (1975) found an increase in the concentration of some metals with animal size, and a decrease in other metals. Thus unless animals are of the same size and physiological condition it is impossible to assess whether differences in tissue metal concentration between two sites reflect real differences in environmental trace element levels or other variations relating to the individual animals. The lower tissue metal levels of larger *Amphibola* occurring on sediments at stations 1 and 2, with higher sediment levels of each metal, however, demonstrate that *Amphibola* does not take up metal ions to a concentration per unit weight directly proportional to the sediment concentration. Within the environmental concentrations occurring at these stations in the Avon-Heathcote Estuary, large *Amphibola* are able to regulate ion uptake and excretion to maintain an acceptable concentration of each metal. In some gastropods metals can be translocated in the shell (Spronk et al 1973) or eliminated by slime secretion (Betzer and Pilson 1974).

The higher level of each metal in the sediment at stations

1 and 2 may indicate higher metal levels in water passing over the sediment or a higher adsorption tendency by the sediment at these two stations. A higher proportion of silt and organic carbon on the western slopes (Chapter 2) would contribute to the accumulation of metal ions in this sediment (Toth and Ott 1970). The levels of metals in the sediment reported in this study for the Avon-Heathcote Estuary, range from relatively low and within the range shown to be characteristic of some unpolluted sediments (Cu 8ppm, Ni 16ppm, Pb 17ppm, Cr 6ppm, Zn 30ppm, Co 6ppm, and Cd 0.4ppm in the Illinois River above a city) for all metals at most sites, to levels of zinc (100.41ppm), copper (24.02ppm) and lead (268.10ppm) in some localised areas in the lower reaches of the Avon River which are comparable to polluted sediments (Cu 19ppm, Ni 27ppm, Pb 28ppm, Cr 17ppm, Zn 81ppm, Co 6ppm, and Cd 2ppm in the Illinois River below a city) (Mathis and Cummings 1973). The order of increasing concentration from nickel to copper to zinc at stations 1 to 4 in this study is identical to that recorded in surface waters and bottom sediments of some aquatic environments in the United States (Toth and Ott 1970). Macpherson (1977) demonstrated that the intertidal slopes around stations 1 to 4 are undergoing net erosion. This erosion will interrupt accumulation of metals and other pollutants in the surface sediments, depending on the residence time of surface silts and organic complexes.

Sediment deposited on the western intertidal slopes around stations 1 and 2 has been shown to arise from the Avon River sediment load rather than the sewage outfalls (Macpherson 1977). Levels of metals reported in the present study for the lower reaches of Avon River suggest that metals may accumulate in

sediments in this area, and be subsequently distributed onto the western estuary slopes in the vicinity of stations 1 and 2. The sediment metal levels in the area of the Bridge Street Bridge are probably a result of dissolved and adsorbed metals from urban run off and wastes carried in the Avon River and concentrated by chemical processes described for low salinity zones of estuaries where metals accumulate after chelation with humic acids (Huggett et al 1973).

The laboratory toxicity experiments showed increased mortality with increase in copper concentration above 1ppm, for *Amphibola* over a 24 day period. Toxicity was further increased by a decrease in salinity below 8‰ sea water and an increase in temperature above 10°C. These affects of a pollutant on a species' ability to adjust to changes in other environmental factors have been reported for other estuarine invertebrates (Vernberg and Vernberg 1972, Jones 1973, 1975a). Temperature, by affecting rate of ionic regulation (Lockwood 1962) affects both the flux of salt ions and toxic metal ions in an estuarine organism. Wide seasonal and daily fluctuations in salinity and temperature within the ranges of levels used in these experiments have been shown for the surface water of the estuarine mud flats at stations 1 to 4 (Chapter II). Increases in concentration of copper above 2.5ppm reduced the survival of *Amphibola* at all levels of temperature, salinity and body size, but had the most affect where the stress from the other environmental factor was least. Thus, for salinity treatments, increase in copper had the greatest effect on survival in 100‰ seawater, which tended to a common LT_{50} plateau with the more dilute seawater treatments. This suggests that copper is not acting directly on *Amphibola* osmotic or ionic regulation sites as

this mode of action would tend to increase the difference in LT_{50} between high and low salinity treatments, as the greatest demands on osmoregulatory systems are at low salinities (Thurberg et al 1973). Other studies on the effects of metals on survival of estuarine organisms at different salinities have examined species in which the gill is the site of respiration and important for ionic and osmotic regulation. In these studies copper has been shown to affect the gill epithelium (Brown and Newell 1972, Scott and Major 1972). In *Amphibola* the predominant respiration surface in the lung, is relatively isolated from the external medium, and this may contribute to the delay in toxicity response of *Amphibola* demonstrated in these experiments. The toxicity to *Amphibola* of zinc, compared with copper, showed a several fold higher toxicity for copper. This is similar to that recorded in a general ranking of metals according to 48h LC_{50} (concentration required to produce a 50% mortality in 48h) for embryos of the American oyster (Waldichuk 1974).

Each life stage of *Amphibola*: egg, larva, juvenile snail and adult, showed a different tolerance to decrease in salinity, and increase in ionic copper. Embryonic development was affected by salinities of 5% seawater while veligers remained active in 3% seawater. With an increase in copper concentration this tolerance pattern was reversed with veligers (0.1ppm) being 10 to 20 times more sensitive than eggs (2.5ppm). A marked difference in tolerance of developing embryos and veliger larvae to different toxicants has been described for bivalves (Davis and Hidu 1969). The increased resistance of the egg may be a result of the surrounding protective capsule. Larvae can be particularly sensitive to a toxicant during brief periods of high growth rate

and activity (Vernberg and Vernberg 1972, Skoog 1973). The toxic level of copper to *Amphibola* larvae (0.1 - 0.5ppm) was slightly higher than that reported by Calabrese et al (1973) for bivalve larvae (0.103ppm), and by Okubo and Okubo (1962) for *Mytilus* larvae larvae (0.1ppm).

In *Amphibola* veligers and small snails showed a higher tolerance to low salinities than eggs and adults. Stages with a high resistance to salinity are generally important dispersal stages (Thorson 1973). It is therefore suggested that veligers and small *Amphibola* are important stages for dispersal through the estuary (see also Chapter IV). The reverse situation has been reported for the mud flat snail, *Nassarius obsoletus* in which large snails survive low salinities better than small snails (Bergmann and Graham 1975) although in general smaller and younger individuals of estuarine species distribute themselves in lower salinity water and migrate seawards as they grow larger (Gunter 1961). The higher resistance to low salinity and lower resistance to increase in copper concentration by the egg, as compared with larvae, was also reflected by the reversal in resistance pattern between small and large snails. Large snails were more resistance to copper (2.5ppm) than small snails (1ppm) which may be a result of a higher growth rate, surface area, or shell permeability in juveniles. The toxicity of metals is related to rates of absorption (Bryan 1971) and to the presence of active sites for binding in enzyme systems and membranes, and may increase or decrease with size of organism depending on the proportion of blind to active binding sites (Jugo 1977), permeability of the gut and body wall and rates of excretion and growth (Raymont and Shields 1963). There is an indication that large animals (above 3g) may be more

sensitive to copper than smaller adults. This may be a result of damaged opercula which were observed in many of the largest individuals at station 1. The relatively low sediment metal-levels at stations 1 and 2; the small difference in survival times between animals 10 to 15mm long; and the absence of an increase in resistance with increase in size (by weight) for animals greater than 15mm, indicate that heavy metals are not the cause of the bimodal frequency distribution, and relatively sparse occurrence of adult snails at stations 1 and 2.

The LT_{50} times reported in this study for *Amphibola* exposed to copper (II) ion levels of 2.5 to 10ppm, show that *Amphibola* has a higher short term resistance to metal levels which normally produce a high 48h mortality in estuarine organisms (Waldichuk 19 1974, Eisler and Gardner 1973) than has been reported previously in the literature. Brief exposure to a high concentration of copper, however, produced an irreversible effect within 12h on *Amphibola* which led to its eventual death. This was exacerbated by salinity stress, but was still expressed at a salinity (10‰) which caused no mortality in controls. This initial damage by copper to *Amphibola* may be similar to that described by Scott and Major (1972) who concluded that when the blue mussel is exposed to 0.3ppm copper(II) the lethal damage occurs during the first 36h and is essentially irreversible. They suggested that the damage is caused by free copper(II) ions. In concentrations of copper below the toxicity level for a species, the individuals are able to detoxify the metal by binding it, for example by mucous secretion. The metal can then be accumulated to extra-ordinarily high levels without apparent effect. In *Amphibola* there appeared to be two thresholds affecting subsequent survival;

The first within the initial 12h, and a second between 24 and 48h. The delay between the two thresholds may be caused by a layer of mucous-bound copper produced during the initial 12h, which inhibits further toxic effect until this layer deteriorates and slime producing cells become exhausted or disrupted by free copper ions. This experiment, subjecting *Amphibola* to high concentrations of a toxicant for a brief period, provides an indication of the possible effect of brief high toxicity waste discharges which have been reported in the Avon and Heathcote Rivers.

Although the levels of ionic copper used in these toxicity experiments were much higher than those which would occur in the environment of *Amphibola* they may allow an experimental assessment of the ability of *Amphibola* to withstand stress from pollutants discharged from outfalls and drains, around which this species is often found. The tolerance of *Amphibola* to copper levels up to 1ppm, implies a high resistance to the effects of pollution, compared with other estuarine benthic invertebrates. This tolerance is probably largely conferred by its pulmonary respiration, and operculum, and its ability to produce large amounts of mucous may be important. Invertebrates respiring with gills need to continuously circulate the surrounding water over membranes which are particularly vulnerable to toxicants, and which though able to produce mucous to bind metal ions, become less functional as a result of this protective action. *Amphibola* is able to retract sensitive tissues, and reduce toxicant levels against membranes by mucous binding. Having evolved on estuarine muds (Watters 1964) which accumulate higher levels of metals and hydrocarbons from natural sources than do most environments, *Amphibola* may have also developed an ability to regulate

internal metal ion levels. It is concluded that short term toxicity studies are misleading for a pulmonate gastropod such as *Amphibola* but that when longer exposure times are used the extent of its resistance, its response with time, and the affect of stress from other environmental factors such as salinity, can be established.

CONCLUSIONS

1. SIZE DISTRIBUTION AND SEWAGE OUTFALLS

A sparse adult population of *Amphibola crenata* on the western slopes of the Avon-Heathcote Estuary in the vicinity of the sewage outfalls, provided a striking contrast with a dense adult population on the upper eastern slopes which were isolated from possible effects of sewage. Previous workers attributed this difference in size distribution to a toxic effect of the sewage effluent. On the basis of the following findings it is concluded in this study, that sewage discharge does not determine the characteristic size distribution of *Amphibola* on the upper western slopes of the estuary, and that it is unlikely that the sparsely distributed large adults in this area are under stress from toxic substances in the sewage effluent:

(1) Sediment on the western slopes had a slightly higher silt and organic content, than that on the eastern slopes. This fine enriched sediment material was supplied predominantly by the sediment load in the Avon River. Freshwater seepage on the upper western slopes decreased salinity levels in the surface sediment during tidal emersion.

(2) These conditions appeared to favour larval settlement. Within the estuary this area around the sewage outfalls provided an environment for settlement, and early growth prior to dispersal to areas suitable to other size classes. The area seemed to be

distinguished by a unique combination of factors, such as particle size, nutrient levels, salinity range, and tidal exposure time, which preferentially attract larval settlement. Small snails showed a high tolerance to low salinity, and a preference for sediment from this area.

(3) In addition to supporting a sparse population of large adults this area supported the highest *Amphibola* productivity, by rapidly growing juveniles, of any area in the estuary. This predominantly juvenile population did not represent a colonising situation, but was the result of an annual cycle of settlement, growth and subsequent dispersal. The same settlement pattern was observed on the eastern slopes but much higher numbers settled on the western slopes. Loss from the study areas was a result of dispersal rather than direct mortality. The dense adult population on the eastern slopes was stable, with growth balanced by emigration, immigration and mortality.

(4) Shell morphology, and ovotestis development were not significantly different between the two areas. These aspects of the biology of *Amphibola* were considered to be possible indicators of sub-lethal stress.

(5) Metal levels in the sediment on the western slopes provide an indicator to all the toxic materials in the sewage which are likely to be accumulated by sediment and benthic organisms. These levels are a function of input from the water passing over the sediment, and adsorption capacity and residue time of the sediment particles. The relatively low levels of heavy metals found in this study therefore indicate that the toxicity of pollutants in the area was unlikely to have a significant effect on resident populations.

(6) Adult *Amphibola* have a high resistance to ionic copper. Juvenile snails, which were more sensitive to copper (and probably to other pollutants) exhibited high growth rates and numbers on the western slopes. Chemical pollution in the overlying water was therefore unlikely to select against intermediate size classes to produce a bimodal size frequency in the area around the sewage outfalls.

11 SIZE DISTRIBUTION AND RESOURCE UTILISATION

An alternative selection mechanism to the toxic effect of sewage effluent is proposed to explain the size frequency differences between the two study areas. In species with a long breeding life, such as *Amphibola* , which can reach high adult densities, it seems particularly advantageous to minimise intraspecific competition between adults and juveniles. An estuary provides a discrete pool with a complex variety of habitats each defined by particular sediment particle size, exposure, salinity, and nutrient components. It is suggested that *Amphibola* has evolved a strategy for partitioning the estuarine environment amongst different size classes such that intraspecific competition is decreased, sexual contract between breeding adults is increased, and the advantages of each benthic habitat are maximally exploited. By concentrating juvenile snails in an area of abundant nutrients without competition from high densities of adults, the high nutritional demands of this life stage can be met, to ensure that they achieve dispersal or reproductive size as rapidly as possible. Utilisation of a complex environment in this way requires dispersal stages, and mechanisms to maintain location once a

particular size class has established itself in an optimal environment. Veliger larvae, and juveniles 5 to 10mm long have been identified as two likely dispersal stages. It is proposed that the morphology of the shell of *Amphibola* , and its burrowing habit reflect the importance to the adult of maintaining a particular position within the estuary.

III SUGGESTIONS FOR FURTHER STUDY

The present study raises many questions and some seem particularly worthy of further investigation:

(1) the factors which affect *Amphibola* larval settlement, the cause of an increase in settlement rate several months after the end of the previous breeding season, the stimuli which control dispersal of juveniles and adults, and the adaptive value of expending energy on dispersion.

(2) the distribution patterns of *Amphibola* in the estuarine environment and the importance of different combinations of factors which determine its spatial distribution and utilisation of habitat areas in an estuary.

(3) the role of the sculpture of the shell, and the interaction between endogenous rhythms and environmental factors which affect shell deposition patterns.

(4) the features of *Amphibola* which confer an especially high resistance to toxic materials, and the mode of action of short term exposure to toxic materials which produces subsequent delayed mortality.

ACKNOWLEDGEMENTS

I wish to especially thank Professor G.A.Knox, and Dr M.B. Jones of the University of Canterbury for their encouragement and interest in this work, and, with Professor J.E. Morton, of the University of Auckland, and Dr J.B.Jillett, and Dr D.D. McGregor of the University of Otago, for their assistance with the preparation of this manuscript. The valuable assistance of the following: the academic and technical staff of the Department of Zoology and Geology, University of Canterbury: the Photographers in the Department of Zoology, University of Canterbury, and the Medical School and Department of Zoology, University of Otago; Adrienne Nathan who helped with heavy metal analyses; Gerald Stokes who helped with histological preparations; Susan Corbett and Frances Bennington who typed this manuscript; and friends who helped with field work, is gratefully appreciated. A Mobil Environmental Grant, for purchase of materials for pollutant analysis, is acknowledged. Finally I wish to express my appreciation to my parents and family for their continuing support and encouragement.

REFERENCES

ANSELL,A.D., 1974. Seasonal changes in biochemical composition of the bivalve *Chlamys septemradiata* from the Clyde Sea Area. Mar. Biol., 25:85-99.

BERGMAN,J.R.& GRAHAM,M.G., 1975. Salinity, a factor defining the habitat of the mud snail, *Nassarius obsoletus* (Say). Proc. malac. Soc. Lond.,41:521-525.

BERRIE,A.D., 1966. Growth and seasonal changes in the reproductive organs of *Lymnaea stagnalis* (L.). Proc. malac. Soc. Lond., 37:83-92.

BERTNESS,M.D., 1977. Behavioral and ecological aspects of shore-level size gradients in *Thais lamellosa* and *Thais emarginata*. Ecology,58:86-97.

BETZER,S.B.& PILSON,M.E.Q., 1974. The seasonal cycle of copper concentration in *Busycon canaliculatum* L. Biol. Bull., 146: 165-175.

BITMAN,J. et al., 1970. DDT-induced inhibition of avian shell gland carbonic anhydrase: a mechanism for thin egg shells. Science, 168:594-595.

BOURGET, E., 1977. Shell structure in sessile barnacles. Naturaliste can., 104:281-323.

BOWEN,H.J.M., 1966. Trace elements in biochemistry. Academic Press, London. 241p.

BOYDEN,C.R., 1974. Trace element content and body size in molluscs. Nature, 251(5473):311-314.

BOYLE,P.R., 1970. Aspects of the ecology of a littoral chiton, *Sypharochiton pellisserpentis* (Mollusca: Polyplacophora). N.Z. J. Mar. Freshwater Res., 4:364-384.

BRIGGS,I.W., 1972. The feeding, ecology and energetics of a population of *Amphibola crenata*. Thesis, M.Sc., University of Auckland.

BROOKS,R.R.& RUMSBY,M.C., 1965. The biogeochemistry of trace element uptake by some New Zealand bivalves. Limnol. Oceanogr., 10:521-527.

BROWN,B.E.& NEWELL,R.C., 1972. The effect of copper and zinc on the metabolism of the mussel *Mytilus edulis*. Mar.Biol., 16(2):108-118.

BRUCE,A., 1953. Report on a biological and chemical investigation of the waters in the Estuary of the Avon and Heathcote Rivers. Christchurch Drainage Board, Christchurch. 45p.

BRYAN,G.W., 1971. The effects of heavy metals (other than mercury) on marine and estuarine organisms. Proc.Roy.Soc.Lond. B., 177:389-410.

BRYAN,G.W.& HUMMERSTONE,L.G., 1975. In: Report of the Council for 1974-75. J.mar.biol.Ass.U.K., 55(4):36-37.

CAIN,A.J., 1977. Variation in the spire index of some coiled gastropod shells, and its evolutionary significance. Phil. Trans.R.Soc., Series B, 277:377-428.

CALABRESE,A., 1972. How some pollutants affect embryos and larvae of American oyster and hard-shell clam. Marine Fish.Review, 34(11-12):66-77.

CALABRESE,A.& NELSON,D.A., 1974. Inhibition of embryonic development of the hard clam, *Mercenaria mercenaria*, by heavy metals. Bull.Environ.Contam.& Toxicol., 11(1):92-97.

CALOW,P., 1975. Length-dry weight relationships in snails: some explanatory models. Proc.malac.Soc.Lond., 41:357-375.

CALOW,P., 1978. The evolution of life-cycle strategies in freshwater gastropods. Malacologia, 17(2):351-364.

CHAPMAN,G., 1949. The thixotropy and dilatancy of a marine soil. J.mar.biol.Ass.U.K., 28(1):123-140.

CHEATUM,E.P., 1934. Limnological investigations on respiration, annual migratory cycle, and other related phenomena in freshwater pulmonate snails. Trans.Am.microsc.Soc., 53:348-407.

COMFORT,A., 1957. The duration of life in molluscs. Proc.malac. Soc.Lond., 32(6):219-241.

COUGHTREY,P.J.& MARTIN,M.H.,1977. The uptake of lead, zinc, cadmium, and copper by the pulmonate mollusc, *Helix aspersa* Muller, and its relevance to the monitoring of heavy metal contamination of the environment. Oecologia(Berl.), 27:65-74.

CROTHERS,J.H., 1975. On variation in *Nucella lapillus*(L.): shell shape in populations from the Channel Islands and North-Western France. Proc.malac.Soc.Lond., 41:499-502.

DALES,R.P., 1952. The larval development and ecology of *Thoracophelia mucronata* (Treadwell). Biol.Bull.mar.biol.Lab., Woods Hole, 102:232-242.

DAVIS,H.C.& HIDU,H., 1969. Effects of pesticides on embryonic development of clams and oysters and on survival and growth of the larvae. Fish.Bull.U.S., 67(2):393-403.

DOUDOROFF,P.& KATZ,M., 1953. Critical review of literature on the toxicity of industrial wastes and their components to fish. II. The metals as salts. Sewage Ind. Wastes, 25:802-839.

DRISCOLL,E.G., 1975. Sediment-animal-water interaction, Buzzards Bay, Massachusetts. J.Mar.Res., 33(3):275-302.

DRUMMOND,J., comp., 1902. Nature in New Zealand. Whitcombe and Tombs Ltd., Christchurch. 188p.

ECKBALD,J.W., 1971. Weight length regression models for three aquatic gastropod species. Am.Midl.Nat., 85:271-274.

EDWARDS,D.C., 1969. Zonation by size as an adaption for intertidal life in *Olivella biplicata*. Am.zoologist, 9:399-417.

EISLER,R., 1970. Latent effects of insecticide intoxication to marine molluscs. Hydrobiologia, 36(3-4):345-352.

EISLER,R.& GARDNER,G.R., 1973. Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts. J.Fish.Biol., 5:131-142.

ESTCOURT,I.N., 1962. An ecological survey of the burrowing polychaetes of the Heathcote-Avon Estuary. Thesis, M.Sc., University of Canterbury.

FAO FISHERIES REPORTS, 1971. Report of the FAO Technical Conference on marine pollution and its effects on living resources and fishing. FAO, Rome, No.99. 188p.

FARNIE,W.C., 1919. The structure of *Amphibola crenata* Martyn. Trans.N.Z.Inst., 51:69-85.

FARNIE,W.C., 1924. The development of *Amphibola crenata* (Martyn). Q.Jl microsc.sci., 68:453-469.

FOLK,R.L., 1966. A review of grainsize parameters. Sedimentology, 6:73-94.

FOLK,R.L., 1968. Petrology of sedimentary rocks. The University of Texas, Hemphill's, Drawer M, University Station, Austin, Texas. 154p.

FRANK,P.W., 1965. Shell growth in a natural population of the turban snail, *Tegula tunebralis*. Growth, 29:395-403.

FRANZ,D.R., 1971. Population age structure, growth and longevity of the marine gastropod *Urosalpinx cinerea* Say. Biol.Bull., 140:63-72.

FRETTER,V.& GRAHAM,A., 1964. Reproduction. In: Physiology of Mollusca, v.1, WILBUR,K.M. & YONGE,C.M., Eds. Academic Press, N.Y., p.127-164.

FRIEDMAN,G.M., 1967. Dynamic processes and statistical parameters compared for size frequency distribution of beach and river sands. J.sedim.Petrol., 37:327-354.

GIESEL,J.T., 1970. On the maintenance of a shell pattern and behavior polymorphism in *Acmaea digitalis*, a limpet. Evolution, 24:98-119.

GILLESPIE,D.M., 1969. Population studies of four species of molluscs in the Madison River, Yellowstone National Park. Limnol.Oceanogr., 14:101-114.

GOLDBERG,E.D., 1965. Minor elements in sea water. In: Chemical Oceanography, v.1, RILEY,J.P. & SKIRROW,G., Eds. Academic Press, N.Y. p.163-196.

GROSS,M.G., 1970. Waste removal and recycling by sedimentary processes. In: Report of the FAO Technical Conference on marine pollution and its effects on living resources and fishing. FAO Fisheries Reports, No.99, p.104.

GUNTER,G., 1961. Some relations of estuarine organisms to salinity. Limnol.Oceanogr., 6(2):182-190.

HARMAN,W.N., 1970. A local case of intra-population variation in *Lymnaea emarginata* (Say). American Malacological Union, Annual Report, p.45-47.

HEWITT,E.J.& NICHOLAS,D.J.D., 1963. Cations and anions: inhibitions and interreactions in metabolism and enzyme activity. In: Metabolic Inhibitors, v.2, HOCHSTER,R.M. & QUASTEL,J.H., Eds. Academic Press, N.Y. p.311-436.

HIDU,H.& HANKS,J.E., 1968. Vital staining of bivalve mollusk shells with alizarin sodium monosulfonate. Proc.natn. Shellfish.Ass., 58:37-41.

HUGGETT,R.J., et al, 1973. Utilizing metal concentration relationships in the eastern oyster (*Crassostrea virginica*) to detect heavy metal pollution. Water Research, 7:451-460.

HUGHES,R.N., 1970. Population dynamics of the bivalve *Scrobicularia plana* (Da Costa) on an intertidal mud-flat in North Wales. J.Anim.Ecol., 39:333-356.

HUXLEY,J.S., 1972. Problems of relative growth. 2d ed. Dover, N.Y. p.151-164.

JOHNSON,R.G., 1974. Particulate matter at the sediment-water interface in coastal environments. J.mar.Res., 32(2):313-330.

JOLLY,J.N., 1971. Feeding and burrowing behaviour of *Amphibola crenata*. Unpub. M.Sc. project in zoology, University of Canterbury, New Zealand.

JONES,M.B., 1973. Influence of salinity and temperature on the toxicity of mercury to marine and brackish water isopods (crustacea). Estuar.coast.mar.Sci., 1:425-431.

JONES,M.B., 1975a. Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine and estuarine isopods (crustacea). Mar.Biol., 30:13-20.

JONES,M.B., 1975b. Effects of copper on survival and osmoregulation in marine and brackish water isopods (crustacea). Proc. 9th Europ.mar.biol.Symp., p.419-431.

JOOSE,J.et al, 1968. Gametogenesis and oviposition in *Lymnaea stagnalis* as influenced by γ-irradiation and hunger. Symp. zool.Soc.Lond., 22:213-235.

JUGO,S.,. 1977. Metabolism of toxic heavy metals in growing organisms: a review. Environ.Res., 13:36-46.

KILNER,A.R., 1969. Distributions of *Amphibola crenata* and *Zeliloma subrostrata* in the Heathcote-Avon Estuary. Unpub. M.Sc. project in zoology, University of Canterbury, New Zealand. 34p.

KISELEVA,M.I., 1979. Characteristics of size composition of bivalve mollusc populations inhabiting different zones of a biotope. Hydrobiol. J.,15:44-47.

KLOVAN,J.E., 1966. The use of factor analysis in determining depositional environments from grain-size distributions. J.sedim.Petrol., 36(1):115-125.

KNOX,G.A.& KILNER,A.R., 1973. The ecology of the Avon-Heathcote Estuary. Unpublished Report to the Christchurch Drainage Board. 358p.

KUBO,I., 1953. Age determination of the *Babylonica japonica* (Reeve) an edible marine gastropod, basing on the operculum. J.Tokyo Univ. Fish. 34:199.

LAMMENS,J.J., 1967. Growth and reproduction in a tidal flat population of *Macoma balthica* (L.). Neth.J.Sea Res., 3(3): 315-382.

LINSLEY,R.M., 1978. Locomotion rates and shell form in the gastropoda. Malacologia, 17(2):193-206.

LOCKWOOD,A.P.M., 1962. The osmoregulation of crustacea. Biol.Rev., 37:257-305.

LUCKENS,P.A., 1970. Variation of shell characters of a species of *Ocenebra* (Mullusca: gastropoda) at Asamushi. Sci.Rep.Tôhoku Univ.Ser.IV(Biol.), 35:149-159.

LUTZ,R.A., 1976. Annual growth patterns in the inner shell layer of *Mytilus edulis* L. J.mar.biol.Ass.U.K., 56(3):723-731.

MACINNES,J.R.& THURBERG,F.P., 1973. Effects of metals on the behaviour and oxygen consumption of the mud snail. Marine Poll.Bull., 4(12):185-186.

McLAY,C.L. et al, 1975. An inventory of the status and origin of New Zealand estuarine systems. Unpub. project in zoology, University of Canterbury, New Zealand, 41p.

MACPHERSON,J.M., 1977. Environmental geology of the Avon-Heathcote Estuary. Thesis, PhD., University of Canterbury, New Zealand.

MALEK,E.A.& CHENG,T.C., 1974. Medical and economic malacology. Academic Press, N.Y.

MARSHALL,N., 1972. Interstitial community and sediments of shoal benthic environments. In: Environmental framework of coastal plain estuaries. NELSON,B.W.,Ed. Mem.geol.Soc.Am., 133:409-415.

MATHIS,B.J.& CUMMINGS,T.F., 1973. Selected metals in sediments, water and biota in the Illinois river. Jour.Water Poll.Control Fed., 45(7): 1573-1583.

MAYNARD SMITH,J., 1971. What use is sex? J.Theor.Biol., 30:319-335.

MEADE,R.H., 1972. Transport and deposition of sediments in estuaries. The Geological Society of America,Inc. Memoir, 133:91-120.

MERLINI,M., 1971. Heavy metal contamination. In:Impingement of man on the oceans. HOOD,D.W., Ed. Wiley,N.Y., p.461-486.

MITROVIC,V.V., 1972. Sublethal effects of pollutants on fish. FAO, Marine Pollution and Sealife, Section 3:252-257.

MORGANS,J.F.C., 1956. Notes on the analysis of shallow-water soft substrata. J.Anim.Ecol., 25:367-387.

MORTON,B., 1975. The seasonal variation in the feeding and digestive cycle of *Amphibola crenata* (Martyn 1784)(Gastropoda: Pulmonata). Forma et Functio, 8(2):175-190.

MORTON,J.E., 1955. The functional morphology of *Otina otis*, a primitive marine pulmonate. J.mar.biol.Ass.U.K., 34:113-150.

MORTON,J.E. pers comm.

MORTON,J.E.& MILLER,M., 1968. The New Zealand sea shore. Collins, London. 638p.

MUNAWAR,M., 1972. Ecological studies of Euglenineae in certain polluted and unpolluted environments. Hydrobiologia, 39(3): 307-320.

NATIONAL ESTUARINE POLLUTION STUDY, 1970. U.S.D.I., Document No.91-58. U.S. Goverment Printing Office, Washington. 633p.

NEWELL,R.C., 1965. The role of detritus in the nutrition of two marine deposit feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macoma balthica*. Proc.Zool.Soc.Lond., 144:25-45.

NEWELL,R.C., 1970. Biology of intertidal animals. London, Logos Press. 555p.

NIELSEN,S.A.& NATHAN,A., 1975. Heavy metal levels in New Zealand molluscs. N.Z.J.Mar.Freshwater Res., 9(4):467-481.

OKUBO,K.& OKUBO,T., 1962. Study on the bio-assay method for the evaluation of water pollution. II. Use of fertilized eggs of sea urchins and bivalves. Bull.Tokai reg.Fish.Res.Lab., 32:131-140.

PAINE,R.T., 1971. Energy flow in a natural population of the herbivorous gastropod *Tegula funebralis*. Limnol.Oceanogr., 16:86-98.

PANNELLA,G.& MACCLINTOCK,C., 1968. Biological and environmental rhythms reflected in molluscan shell growth. Paleontological Society, Memoir 2, p.64-80.

PATRICK,R. et al, 1968. The relative sensitivity of diatoms, snails and fish to twenty common constituents of industrial wastes. Prog. Fish-culturist, 30(3):137-140.

PIANKA,E.R., 1970. On "r" and "K" selection. Am.Nat., 104:592-597.

POSTMA,H., 1967. Sediment transport and sedimentation in the estuarine environment. In: Estuaries. LAUFF,G.H., Ed. Am.Assoc. Adv.Sci.Pub.83:158-179.

PRINGLE,B.H. et al, 1968. Trace metal accumulation by estuarine mollusks. J.sanit.Engng.Div.Am.Soc.civ.Engrs., 94:455-475.

RAUP,D., 1961. The geometry of coiling in gastropods. Proc. Natn.Acad.Sci. U.S.A., 47:602-609.

RAUP,D.M., 1966. Geometric analysis of shell coiling: general problems. J.Paleont., 40(5):1178-1190.

RAVERA,O., 1977. Effects of heavy metals(cadmium, copper,chromium and lead) on a freshwater snail: *Biomphalaria glabrata* Say (Gastropoda, Prosobranchia). Malacologia, 16(1):231-236.

RAYMONT,J.E.G.& SHIELDS,J., 1963. Toxicity of copper and chromium in the marine environment. Int.J.Air Wat.Poll., 7:435-443.

RISEBROUGH,R.W. et al, 1968. Polychlorinated biphenyls in the global ecosystem. Nature 220(5172):1098-1102.

ROSENBERG,D.R., 1963. Report on a biological investigation of the estuary of the Avon and Heathcote Rivers. Christchurch Drainage Board, Christchurch. 13p.

RUSSELL-HUNTER,W.D.& MCMAHON,R.F., 1976. Evidence for functional protandry in a fresh-water basommatophoran limpet, *Laevapex fuscus*. Trans.Am.microsc.Soc., 95:174-182.

SCOTT,D.M.& MAJOR,C.W., 1972. The effect of copper (II) on survival, respiration, and heart rate in the common blue mussel, *Mytilus edulis*. Biol.Bull.,143:679-688.

SEED,R., 1969. The ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. II. Growth and mortality. Oecologia (Berl.)3:317-350.

SHUMWAY,S.E. pers. comm.

SKOOG,G., 1973. Salinity reactions of two fresh water snails from brackish water. Oikos Suppl.,15:253-260.

SOKAL,R.R.& ROHLF,F.J., 1969. Biometry, the principles and practice of statistics in biological research. W.H.Freeman, San Francisco. 776p.

SPRAGUE,J.B., 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and "safe" concentrations. Water Research, 5:245-266.

SPRONK,N. et al, 1973. Copper in *Lymnaea stagnalis*. III. Uptake from fresh water and the role of the shell. Comp.Biochem. Physiol., 45A:257-272.

STEFFENSEN,D.A., 1974. Distribution of *Euglena obtusa* Schmitz and *E.Salina* Liebetanz on the Avon-Heathcote Estuary, Christchurch. Mauri Ora 2:85-94.

STROMGREN,T., 1976. Growth patterns of *Mytilus edulis* in relation to individual variation, light conditions, feeding and starvation. Sarsia 60:25-40.

STRUHSAKER,J.W., 1968. Selection mechanisms associated with intraspecific shell variation in *Littorina picta* (Prosobranchia: Mesogastropoda). Evolution, 22:459-480.

TENORE,K.R., 1972. Macrobenthos of the Pamlico River Estuary, North Carolina. Ecol. Monogr., 42:51-69.

THOMPSON,E.F., 1929. An introduction to the natural history of the Heathcote Estuary and Brighton Beach, Canterbury, New Zealand. A study in littoral ecology. Thesis, M.Sc., University of Canterbury, New Zealand.

THORSON,G., 1946. Reproduction and larval development of Danish marine bottom invertebrates. København. 523p.

THURBERG,F.P. et al, 1973. Effects of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs. Mar.Biol., 23:171-175.

TOTH,S.J.& OTT,A.N., 1970. Characterization of bottom sediments: cation exchange capacity and exchangeable cation status. Environ.Sci. & Technol., 4(11):935-939.

TRIPP,M.R., 1974. Effects of organophosphate pesticides on adult oysters (*Crassostrea virginica*). In: Pollution and physiology of marine organisms. Academic Press, N.Y. p.225-236.

TRUEMAN,E.R.& ANSELL,A.D., 1969. The mechanism of burrowing into soft substrates by marine animals. Oceanogr. & Mar.Biol.Ann.Rev., 7:315-366.

TRUEMAN,E.R.& BROWN,A.C., 1976. Locomotion pedal contraction and extension and the hydraulic systems of *Bullia* (Gastropoda: Nassaridae). J.Zool.Lond., 178:365-384.

VALENTINE,D.W. et al, 1973. Asymmetry analysis in fishes: a possible statistical indicator of environmental stress. Fishery Bull.Fish Wildl.Serv.U.S., 71(2):357-370.

VERMEIJ,G.J., 1971a. The geometry of shell sculpture. Forma et Functio, 4:319-325,

VERMEIJ,G.J., 1971b. Gastropod evolution and morphological diversity in relation to shell geometry. J.Zool., 163:15-23.

VERMEIJ,G.J., 1972. Intraspecific shore-level size gradients in intertidal molliscs. Ecology, 53:693-700.

VERMEIJ,G.J., 1973a. Morphological patterns in high-intertidal gastropods: adaptive strategies and their limitations. Mar. Biol., 20:319-346.

VERMEIJ,G.J., 1973b. West Indian molluscan communities in the rocky intertidal zone: a morphological approach. Bull.Mar. Sci., 23(2):351-386.

VERNBERG,W.B.& VERNBERG,F.J., 1972. The synergistic effects of temperature, salinity and mercury on survival and metabolism of the adult Fiddler Crab, *Uca pugilator*. Fishery Bull.Fish Wildl.Serv.U.S., 70:415-420.

VERNBERG,W.B.& VERNBERG,F.J., 1975. The physiological ecology of larval *Nassarius obsoletus* (Say). Proc. 9th Europ.mar.biol. Symp., p.179-190.

VOLLER,R.W., 1973. Salinity, sediment, exposure and invertebrate macrofaunal distributions on the mudflats of the Avon-Heathcote Estuary, Christchurch, New Zealand. Thesis, M.Sc., University of Canterbury, New Zealand.

WADA,K., 1961. Crystal growth of molluscan shells. Bull.Natl. Pearl Research Lab., 7:703-828.

WAKEEL,S.K.& RILEY,J.P., 1956. The determination of organic carbon in marine muds. J.Cons.perm.int.Explor.Mer.,22:180-183.

WALDICHUK,M., 1969. Effects of pollutants on marine organisms: improving methodology of evaluation - a review of the literature. J.Water Poll. Control Fed., 41(9):1586-1601.

WALDICHUK,M., 1974. Some biological concerns in heavy metals pollution. In: Pollution and physiology of marine organisms. Academic Press, N.Y. p.1-57.

WATABE,N. et al, 1958. Studies on shell formation.VIII Electron Microscopy of crystal growth of the nacreous layer of the oyster *Grassostrea virginica*.J.Biophys.Biochem.Cytol., 4:281-286.

WATTERS, P.A., 1957. The ecology and variation of *Amphibola crenata* (Mart.) in the Dunedin area. Thesis, M.Sc., University of Otago, New Zealand.

WATTERS,P.A., 1964. Distribution of *Amphibola crenata* (Pulmonata) in the Dunedin area, with notes on the probable origin of the species. Tran.R.Soc.N.Z.,Zool, 4(4):117-134.

WEBB,B.F., 1965. Report on a biological investigation of the Estuary of the Avon and Heathcote Rivers. Christchurch Drainage Board, New Zealand.

WEBB,B.F., 1873. Fish populations of the Avon-Heathcote Estuary. 3. Gut contents. N.Z. J.Mar.Freshwater Res., 7(3): 223-234.

WIGHAM,G.D., 1975. Environmental influences upon the expression of shell: form in *Rissoa parva* (Da Costa). [Gastropoda: Prosobranchia]. J.mar.biol.Ass.U.K., 55:425-438.

WILBUR,K.M., 1960. Shell structure and mineralisation in molluscs. In: Calcification in Biological Systems. SOGNNAES,R.F. Ed. p15-40. Am.Assoc.Advance Sci.,Washington,D.C.

WILBUR,K.M., 1964. Shell formation and regeneration. In: Physiology of Mollusca, v.1, WILBUR,K.M. & YONGE,C.M. Eds. Academic Press, N.Y., p.243-282.

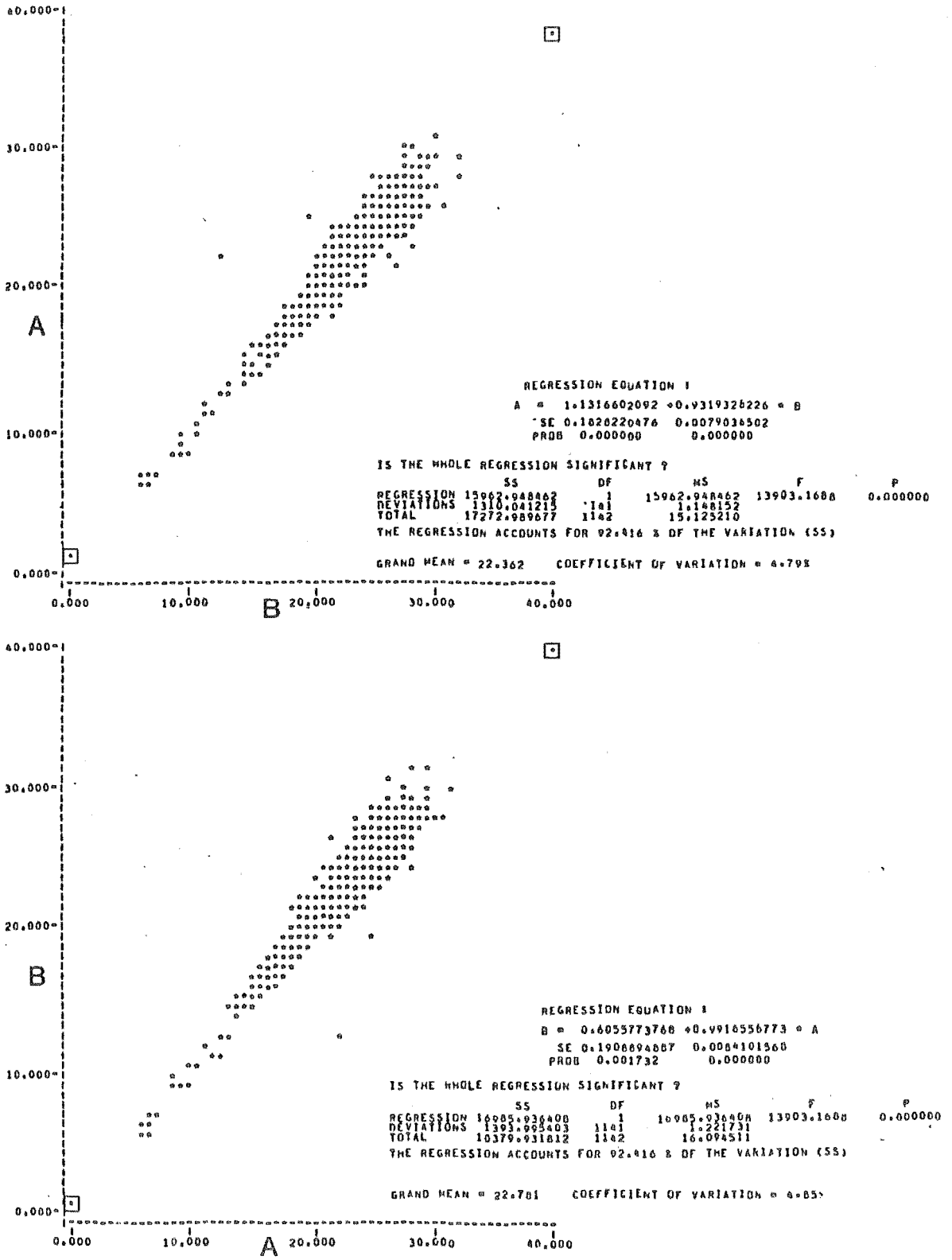
WILBUR,K.M.& OWEN,G., 1964. Growth. In: Physiology of Mollusca, v.1, WILBUR,K.M. & YONGE,C.M. Eds. Academic Press, N.Y., p.211-241.

WILLIAMS,M., 1960. Report on a biological investigation of the estuary of the Avon and Heathcote Rivers, Christchurch, New Zealand. Christchurch Drainage Board, Christchurch. 18p.

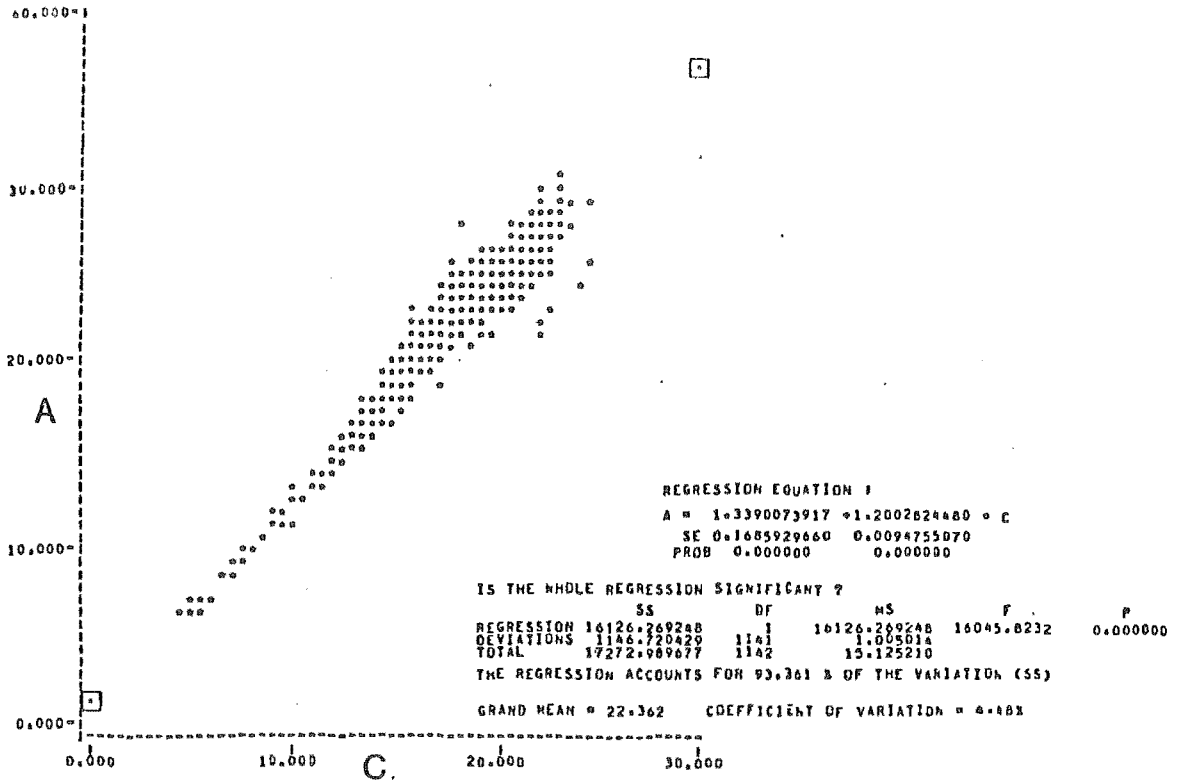
YEVICH,P.P.& BARRY,M.M., 1969. Ovarian tumors in the Quahog *Mercenaria mercenaria*. J.Invertebr.Pathol., 14(2):266-267.

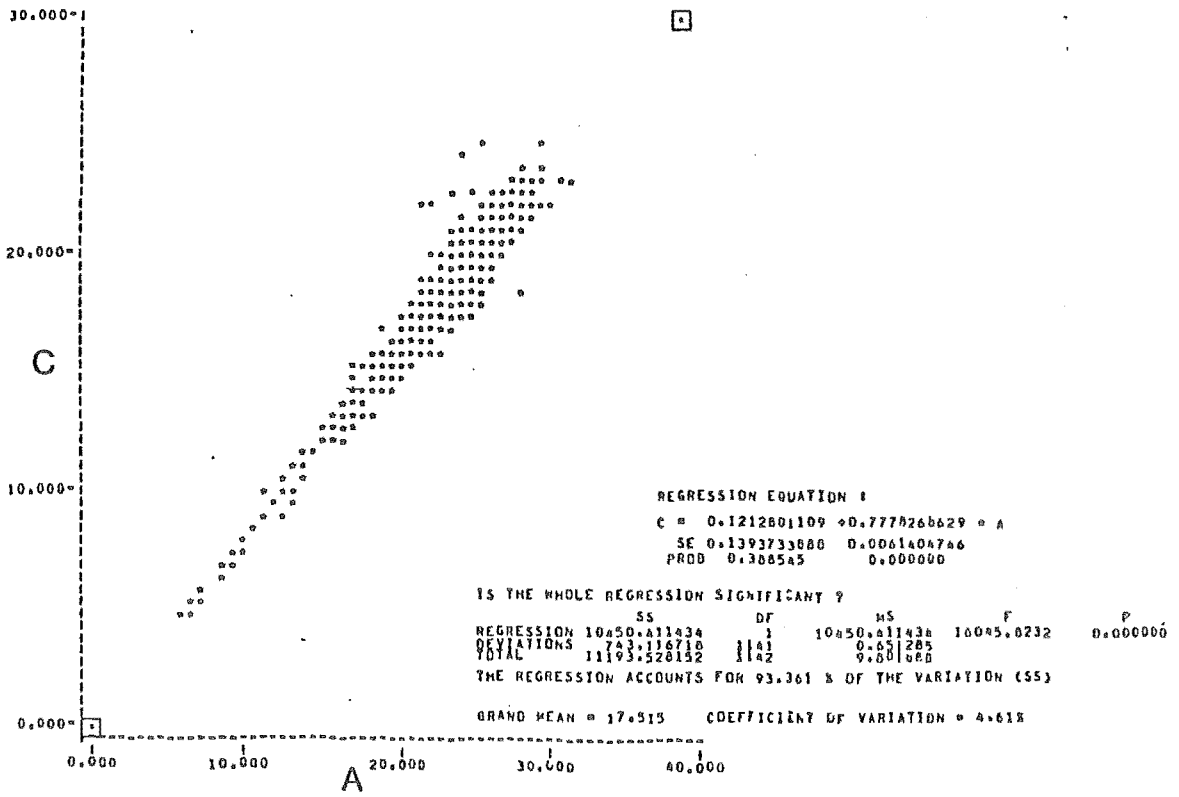
APPENDIX I

REGRESSION LINES OF PAIRS OF VARIATES WHICH DESCRIBE SHELL MORPHOLOGY

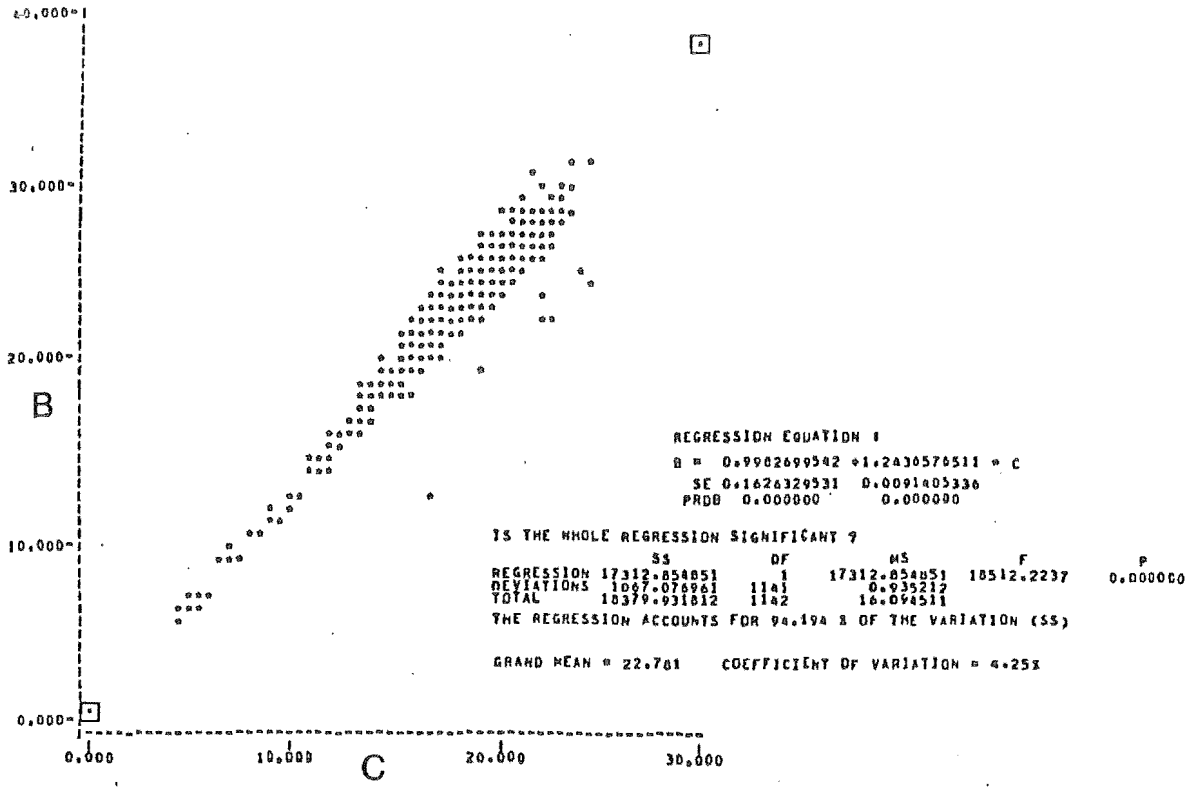


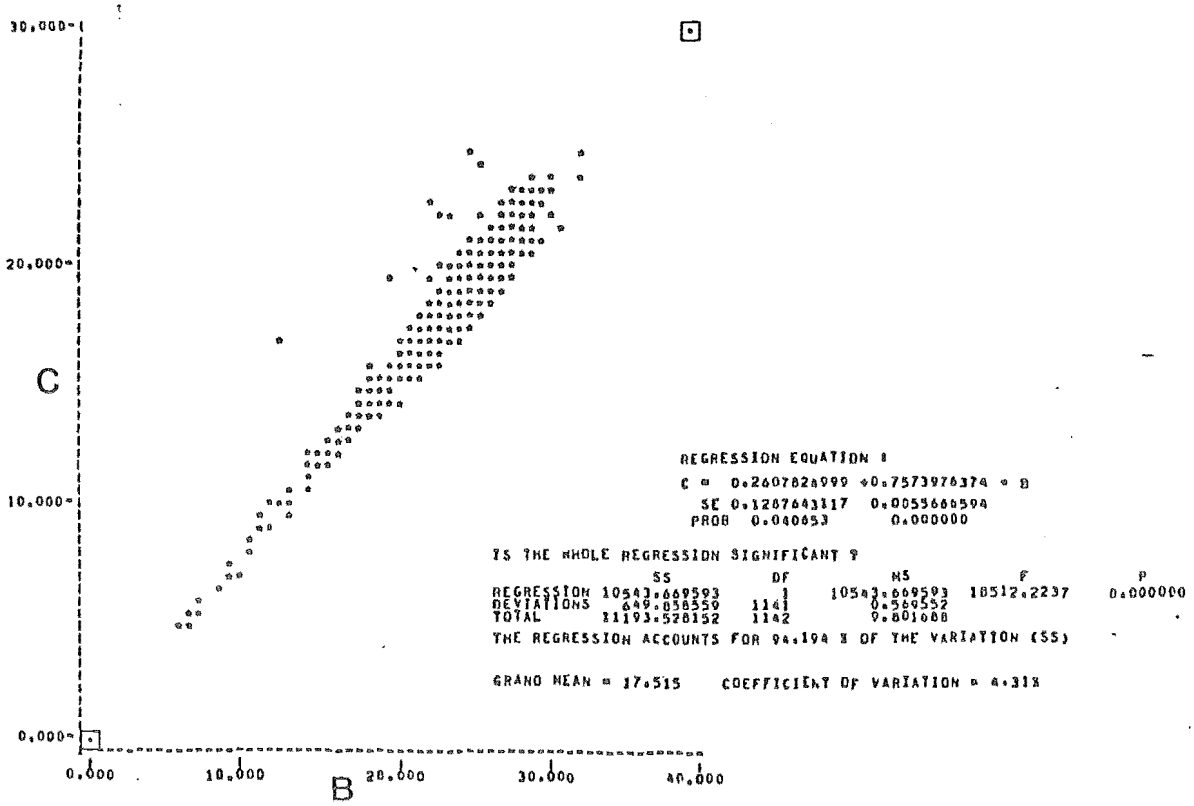
Appendix I.1. Shell length (A) plotted against maximum shell width (B) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



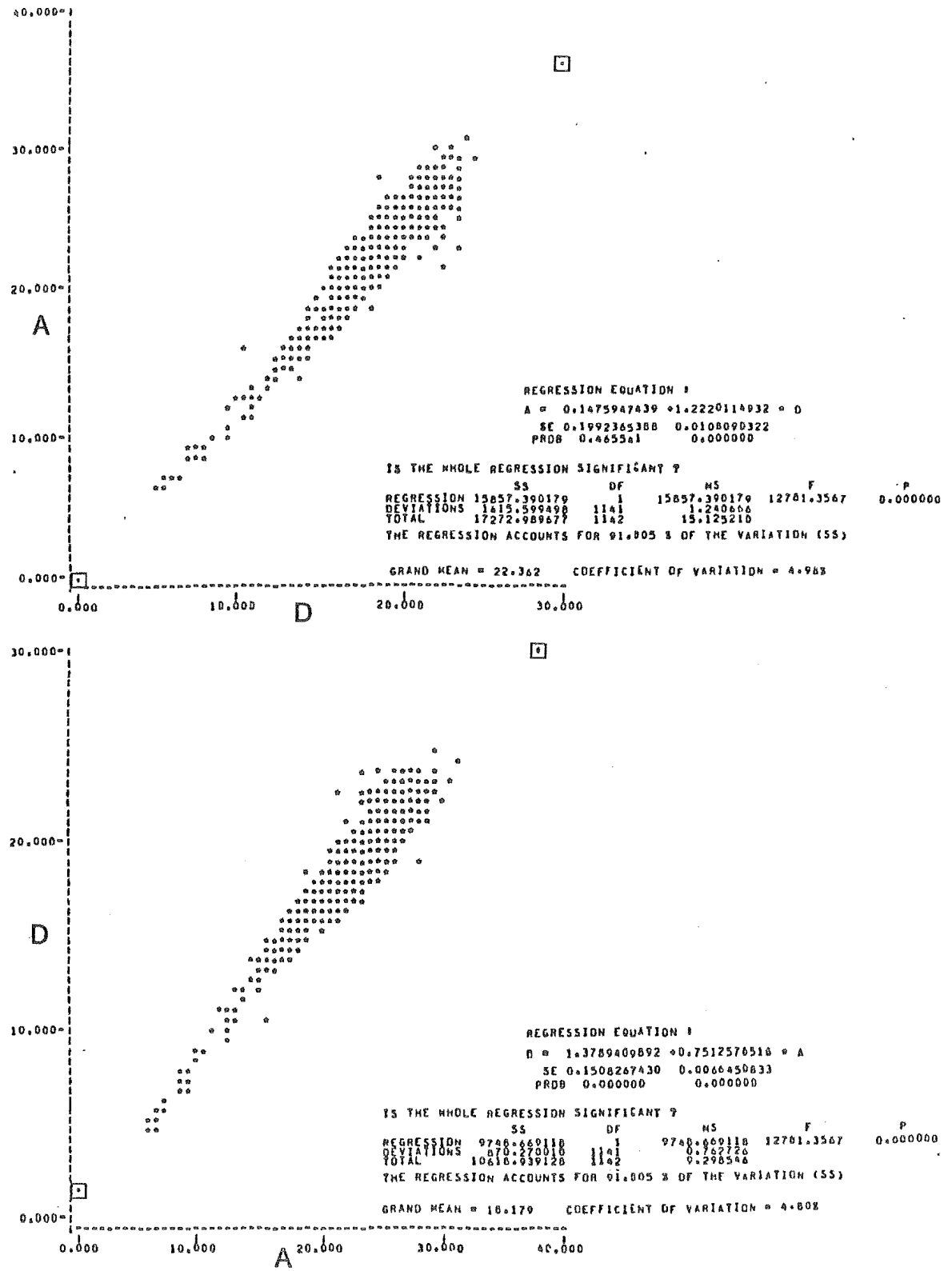


Appendix I.2. Shell length (A) plotted against minimum shell width (C) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.

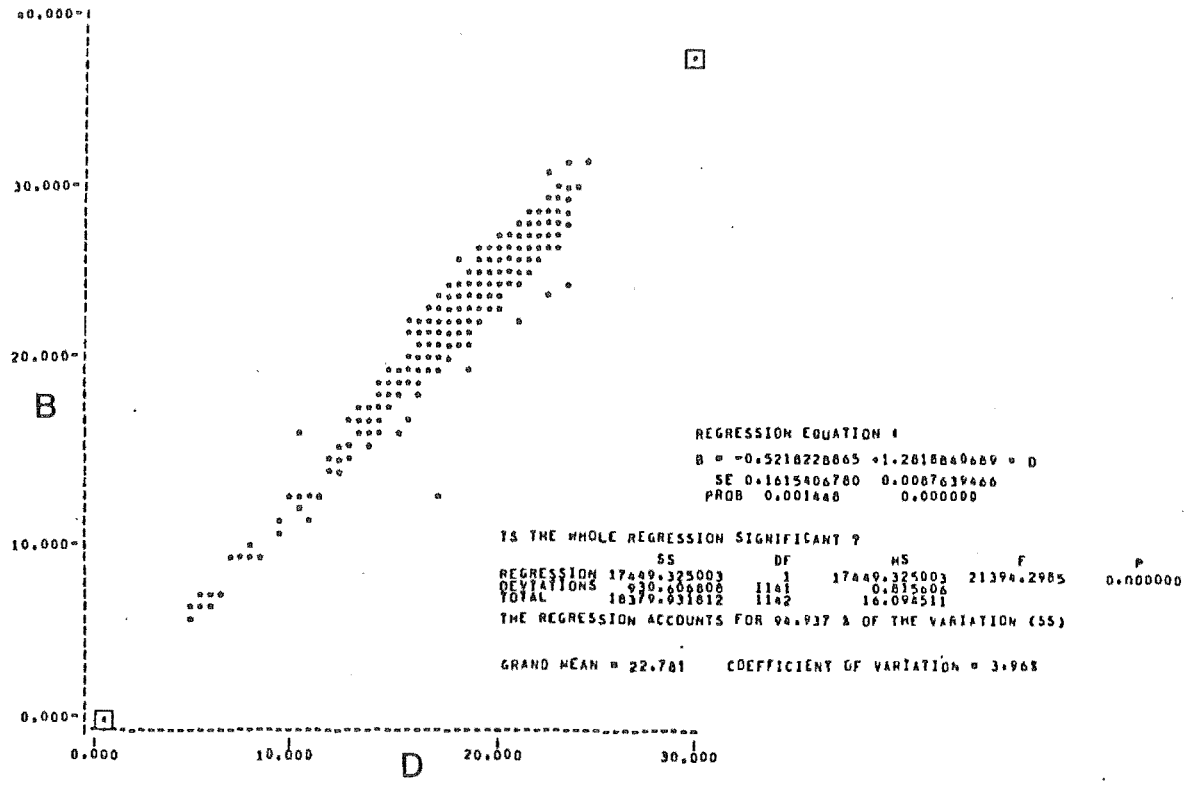


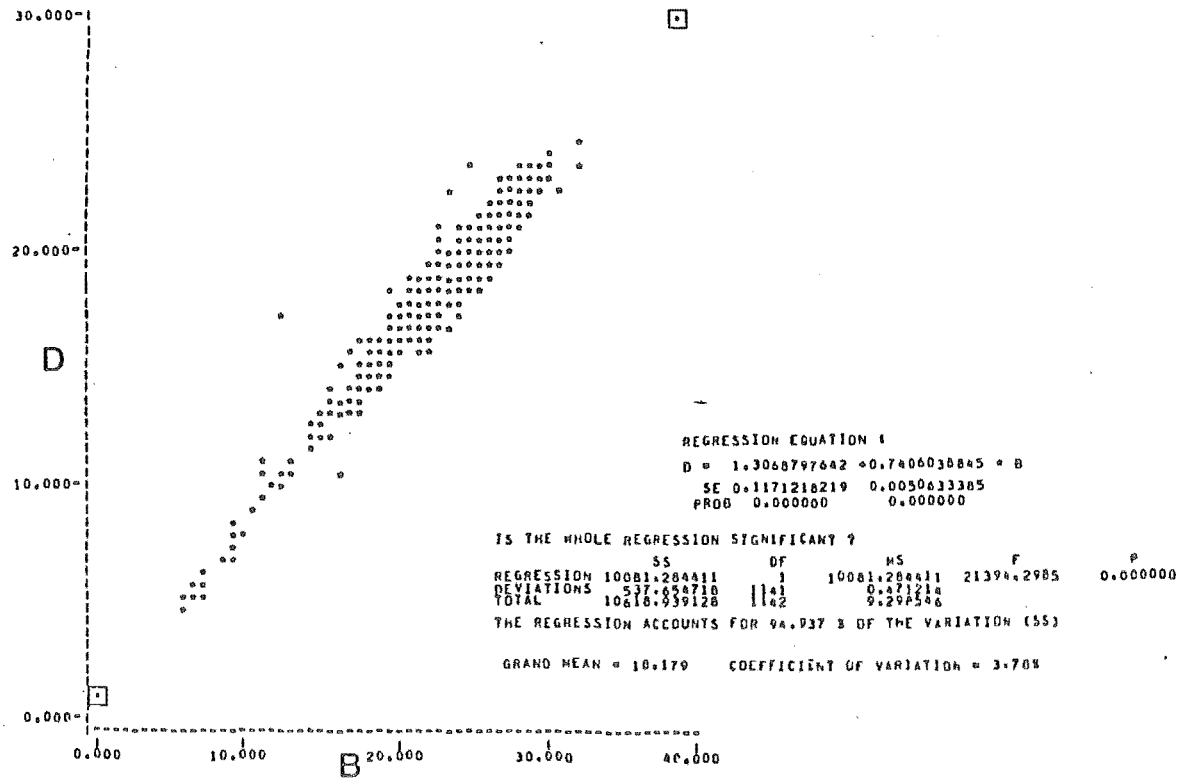


Appendix I.3. Maximum shell width (B) plotted against minimum shell width (C) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.

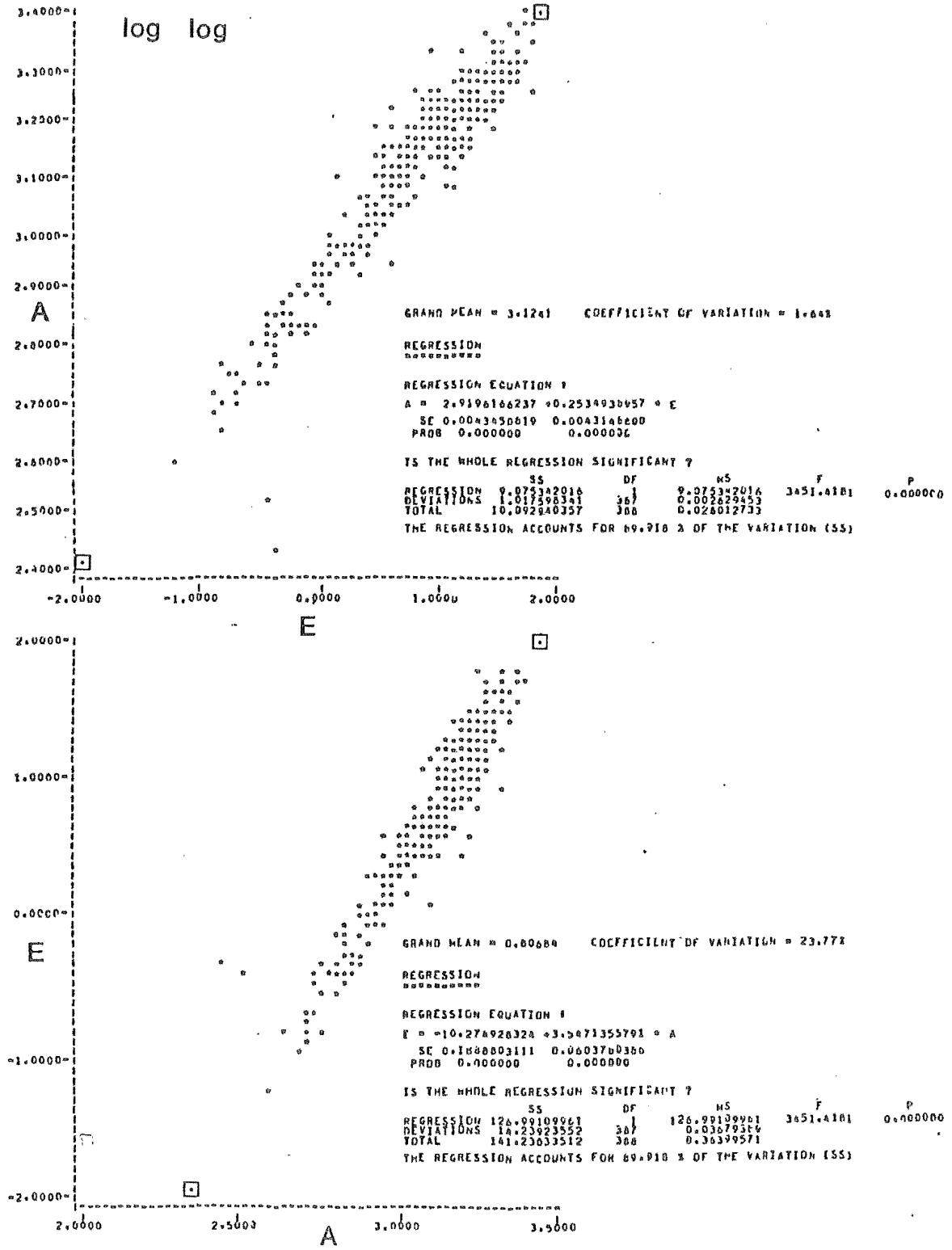


Appendix I.4. Shell length (A) plotted against aperture length (D) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.

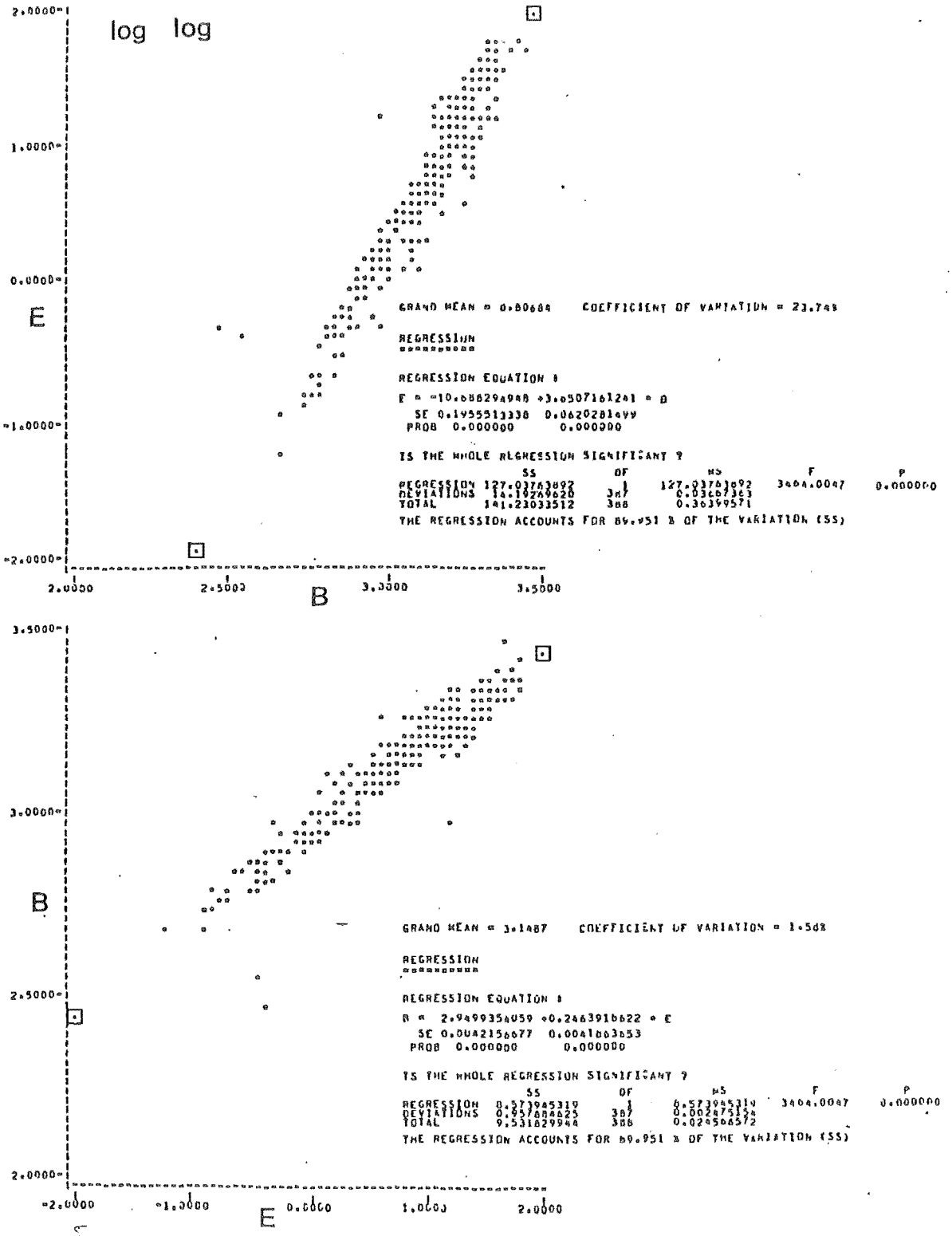




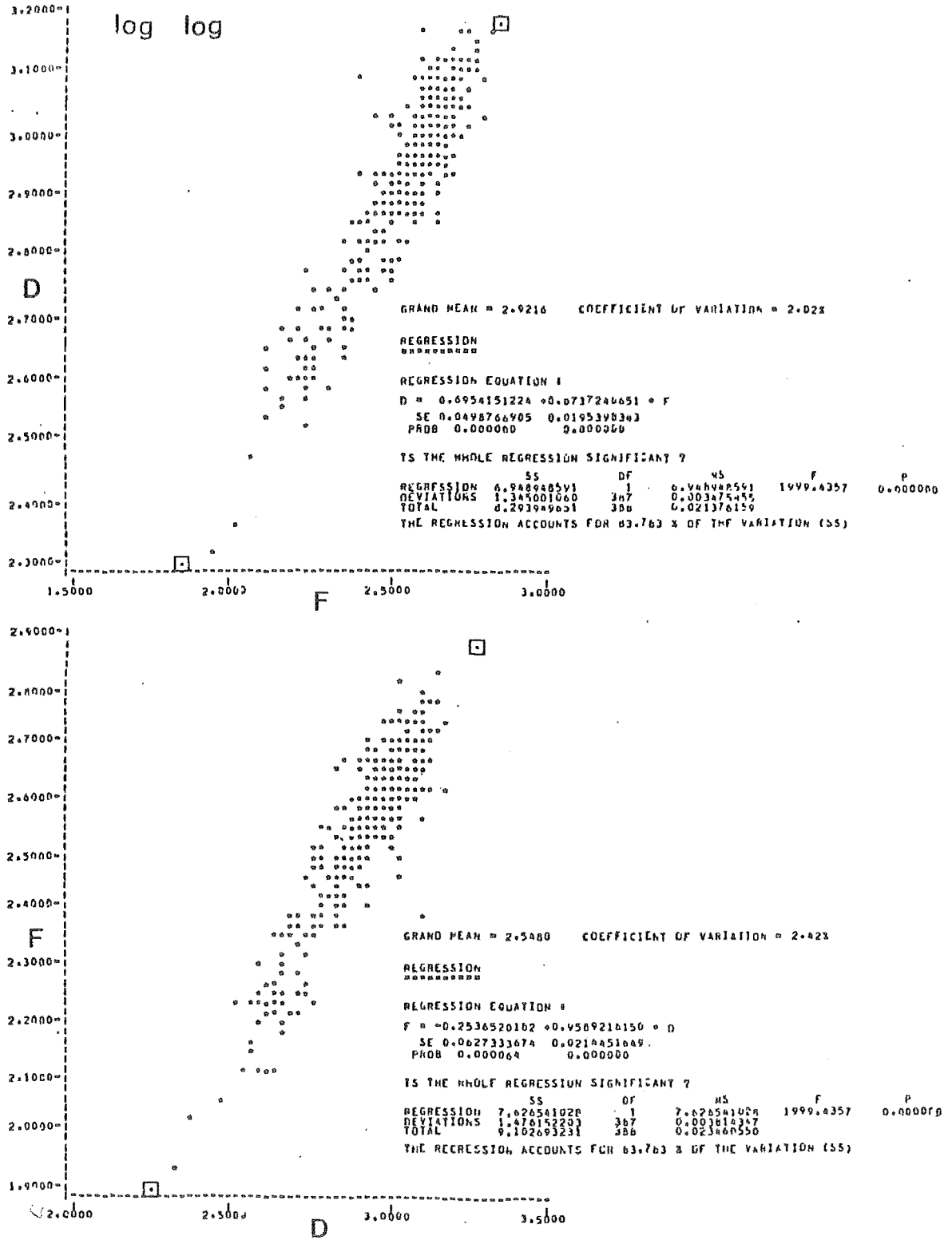
Appendix 1.5. Maximum shell width (B) plotted against aperture length (D) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



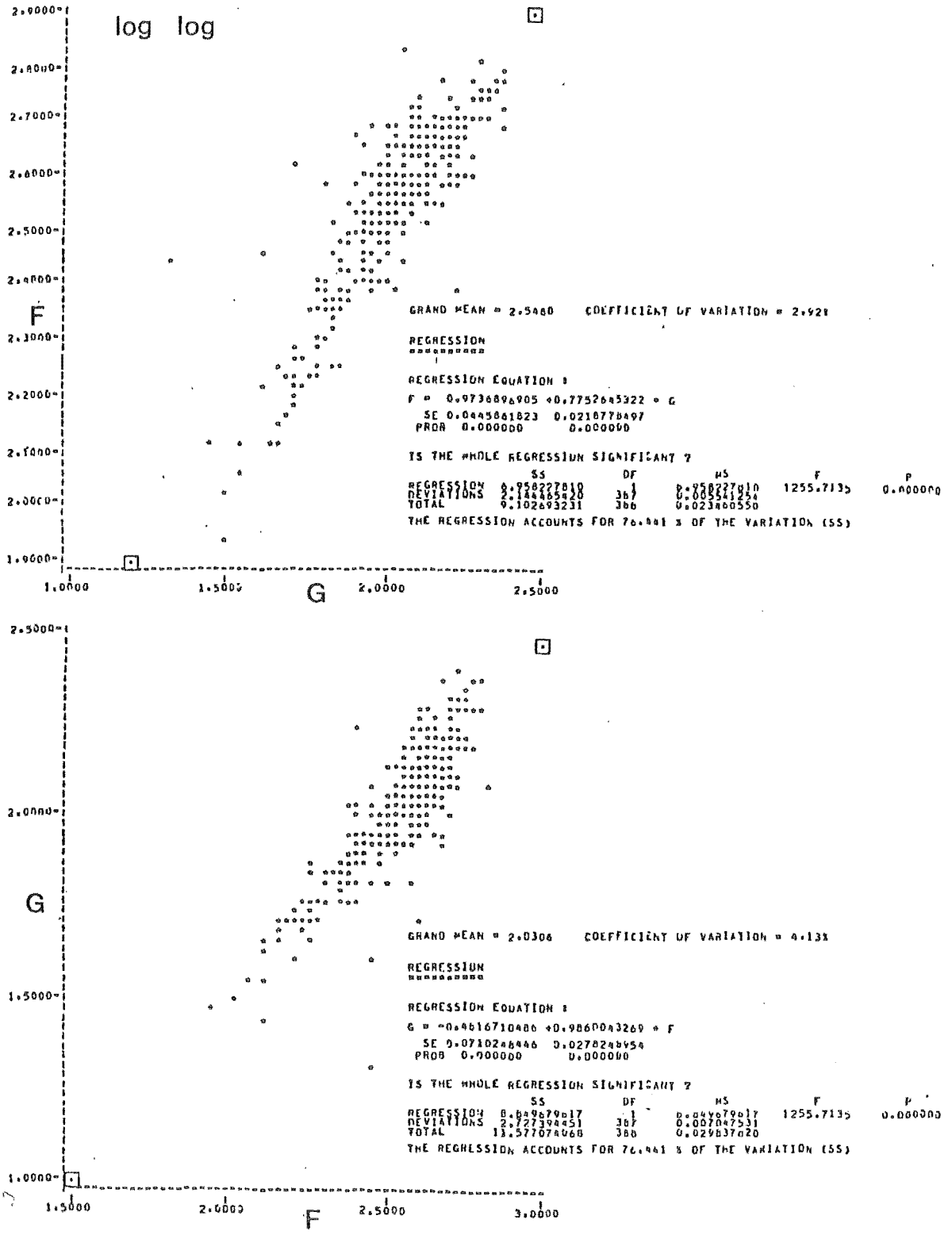
Appendix 1.6. Log shell length (A) plotted against log dry weight (E) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



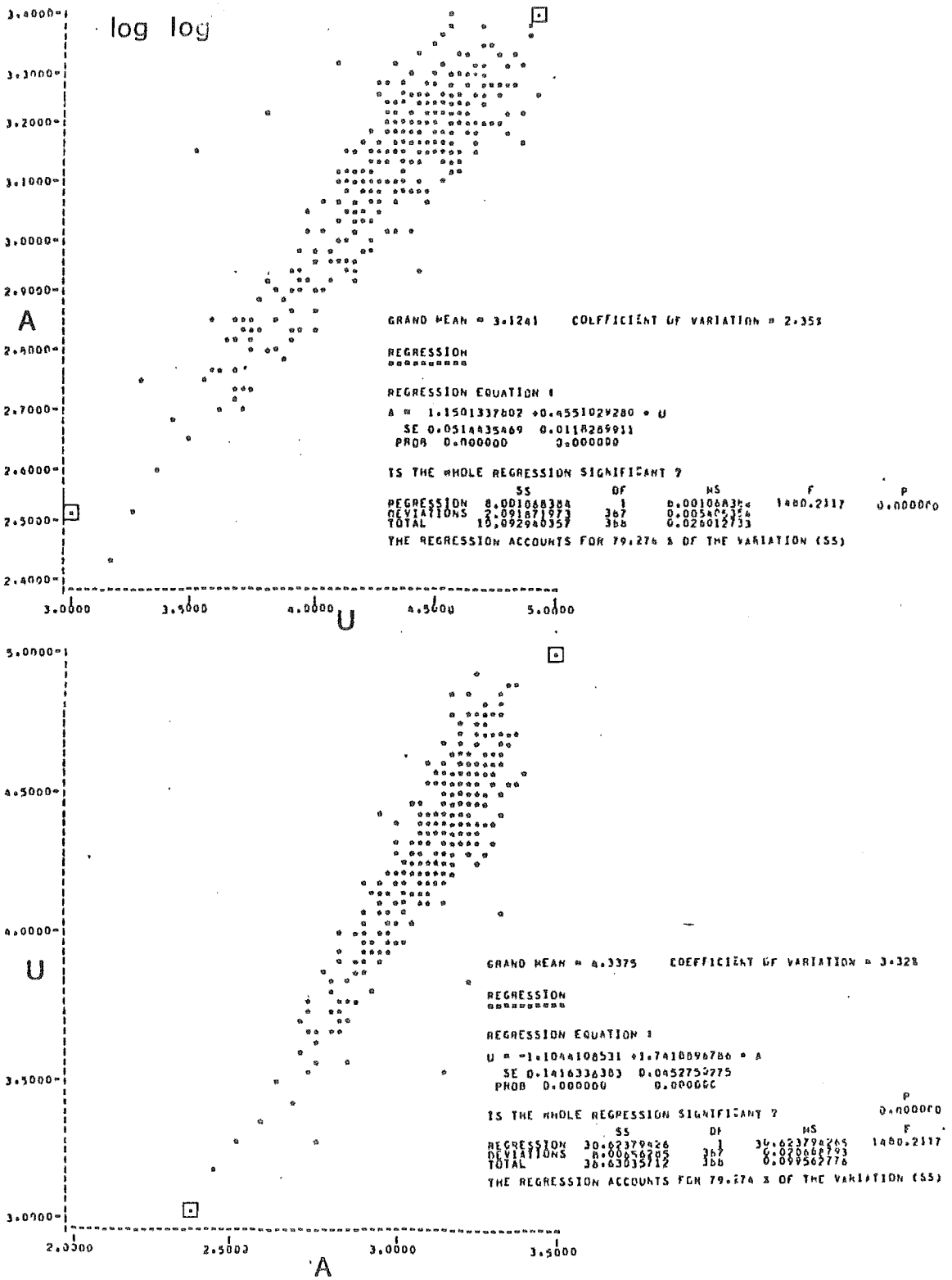
Appendix I.7. Log maximum shell width (B) plotted against log dry weight (E) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



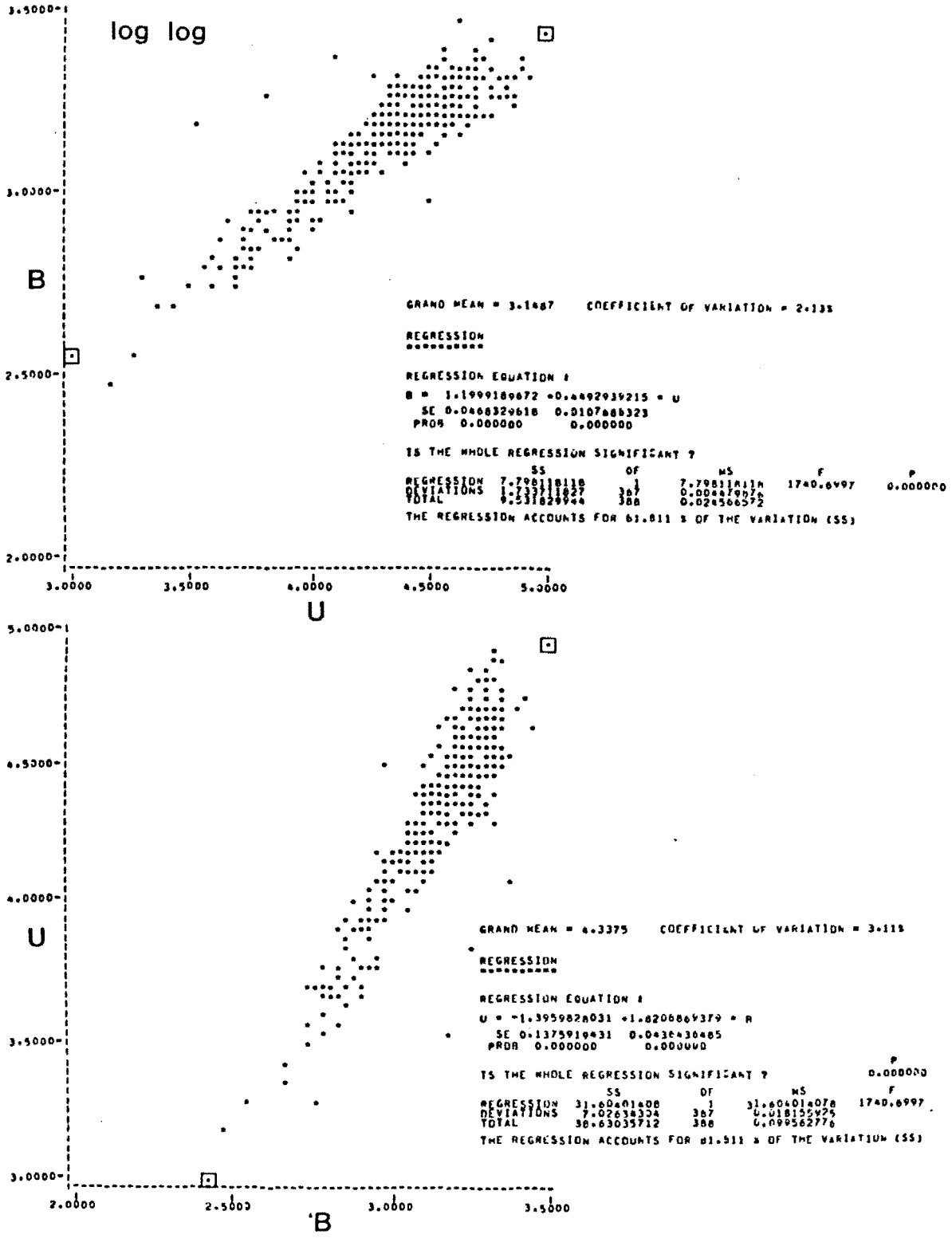
Appendix 1.8. Log aperture length (D) plotted against log operculum length (F) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



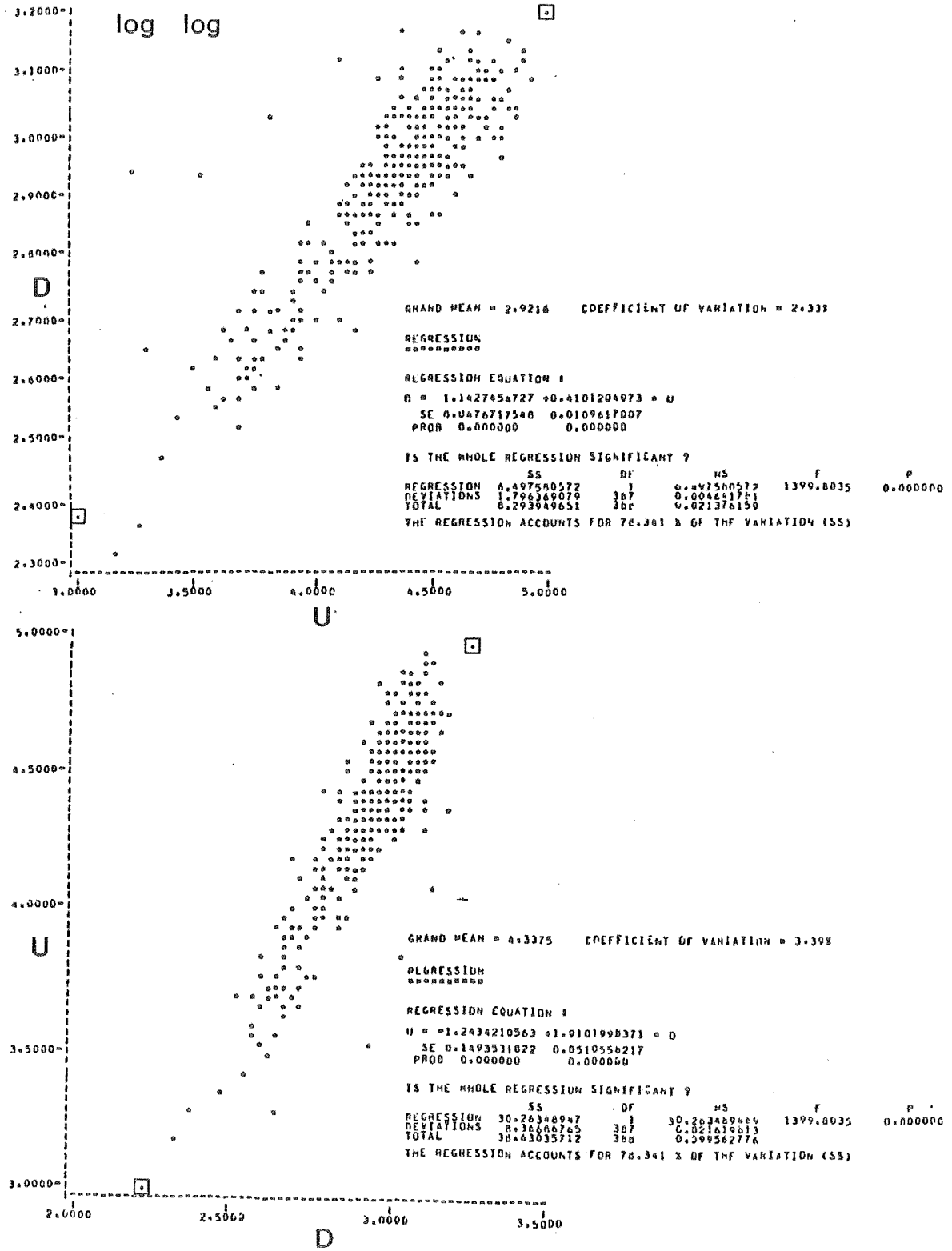
Appendix 1.9. Log operculum length (F) plotted against log operculum width (G) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



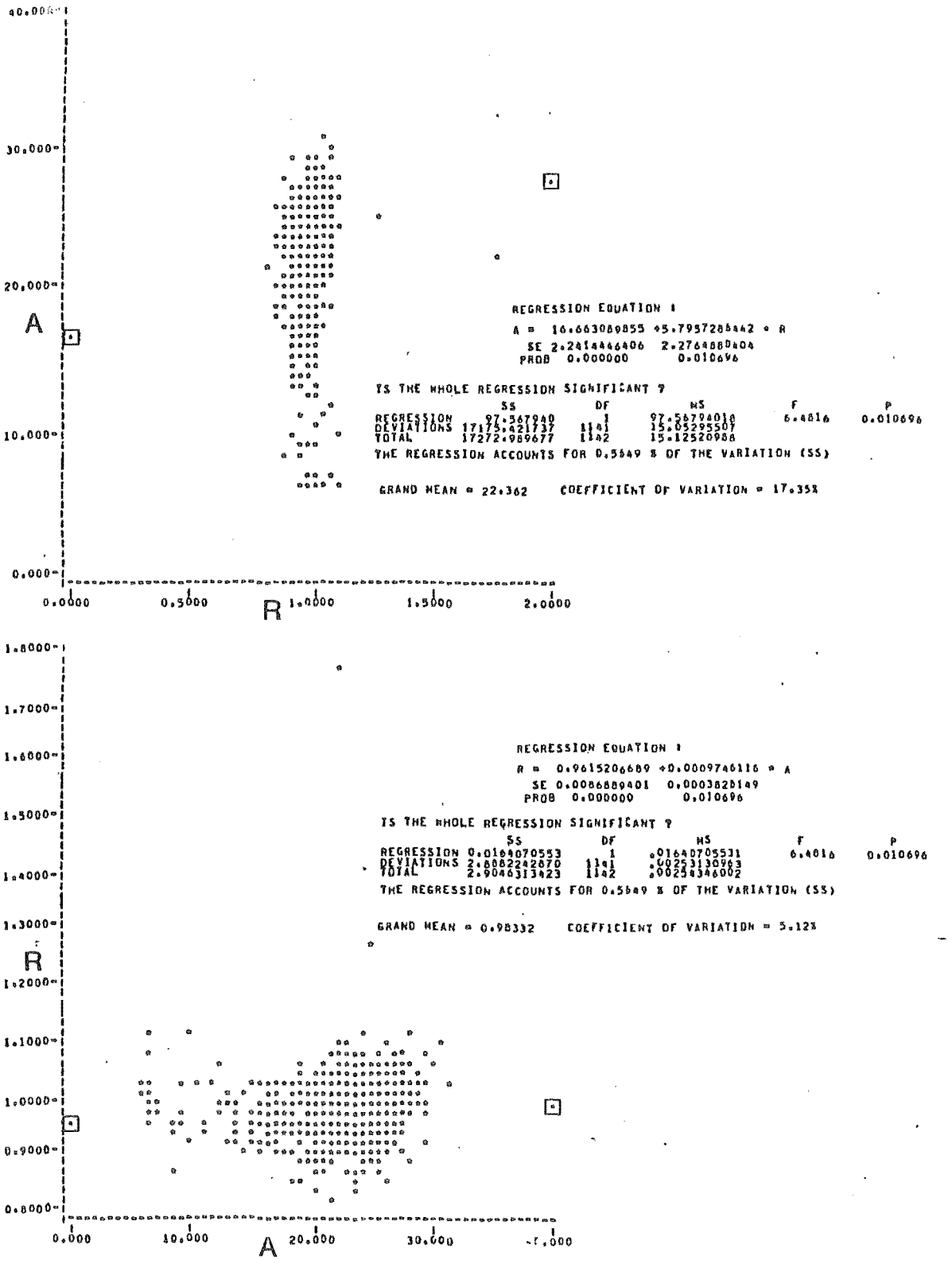
Appendix 1.10. Log shell length (A) plotted against log operculum area (U) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



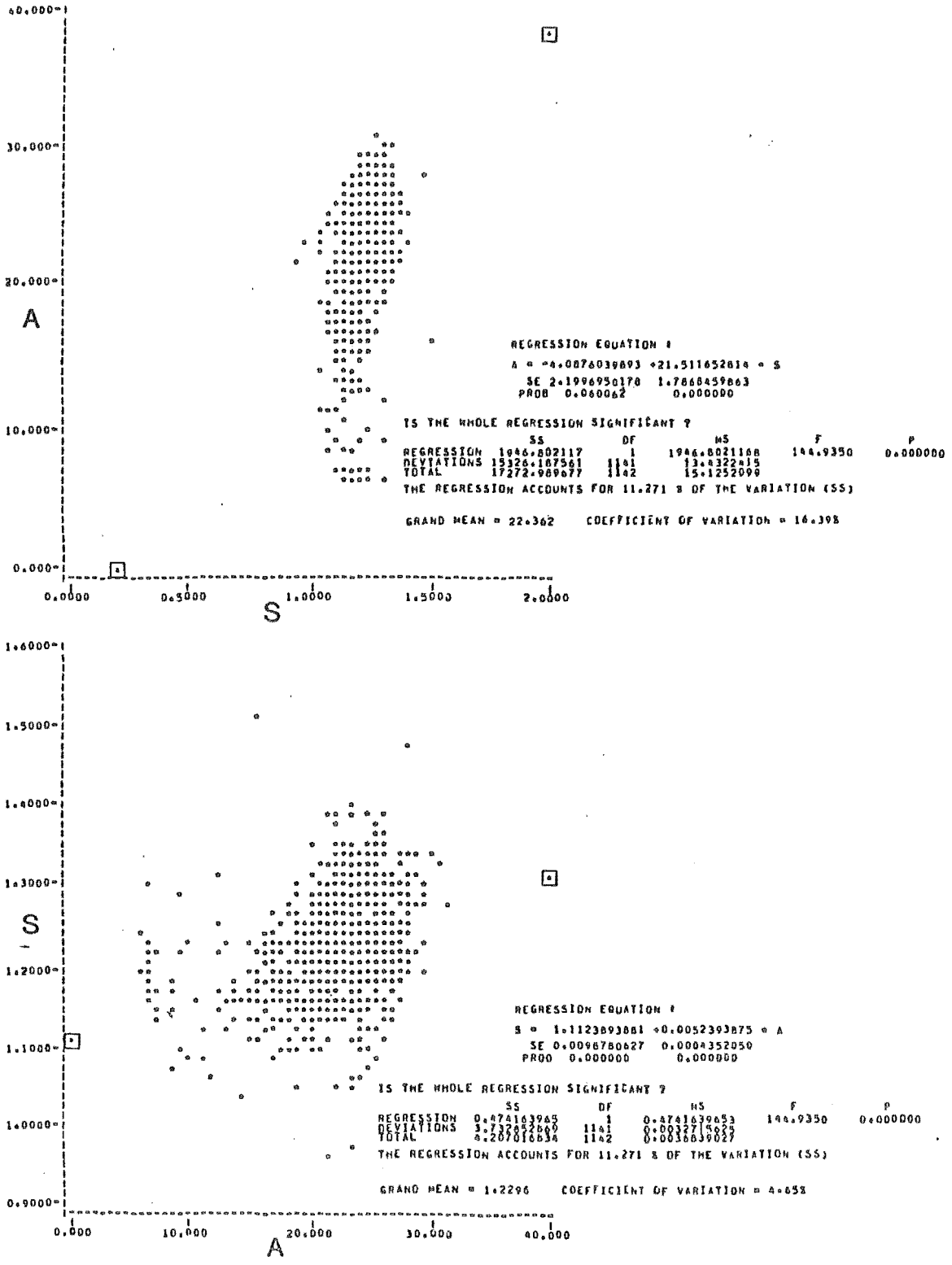
Appendix I.11. Log maximum shell width (B) plotted against log operculum area (U) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



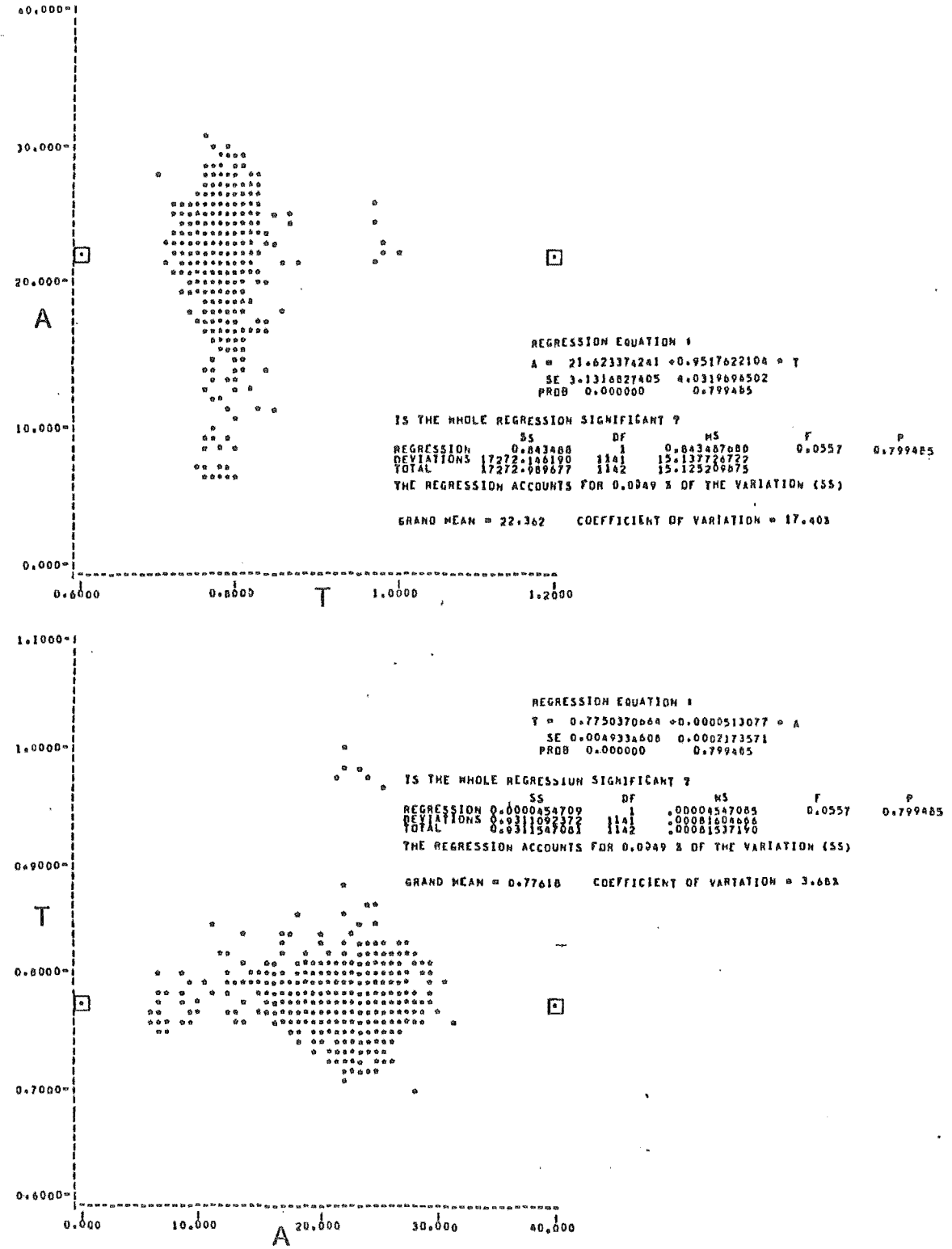
Appendix I.12. Log aperture length (D) plotted against log operculum area (U) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



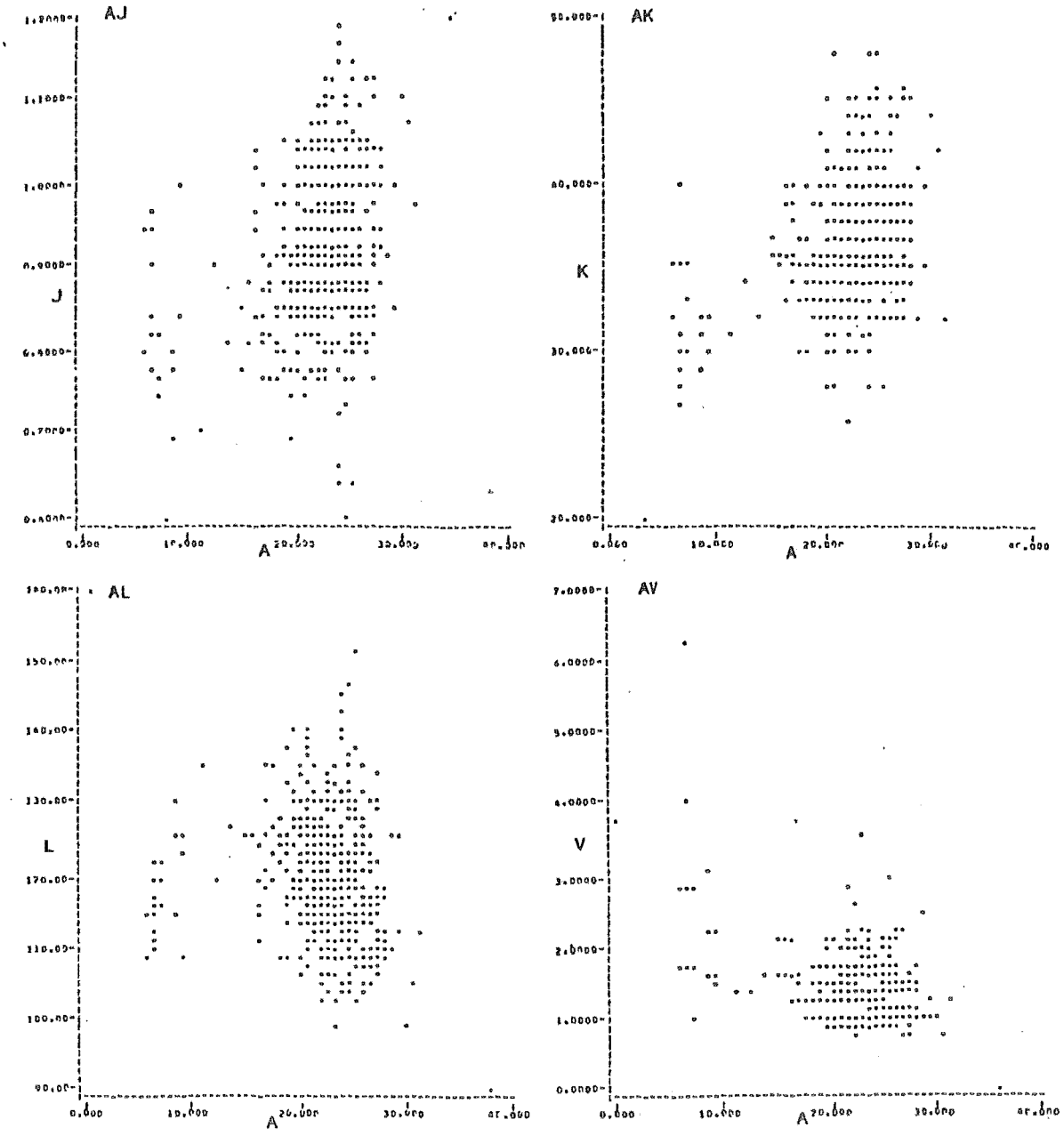
Appendix I.13. Shell length (A) plotted against spire index (R = A/B) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



Appendix I.14. Shell length (A) plotted against (S = A/D) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



Appendix I.15. Shell length, (A) plotted against shell globosity ($T = C/(AxB)^{\frac{2}{3}}$) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.



Appendix I.16. Shell length (A) plotted against translation rate (J = cot apical angle/2), angle of elevation (K), angle at the shell spire (L), and expansion rate of the coils (V = $(H/I)^2$) for *Amphibola crenata* collected at stations 1 to 4, in 1974 and 1975.